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## OATS AND OAT PRODUCTS

### CULTURE, BOTANY, SEED STRUCTURE, MILLING, COMPOSITION, AND USES

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Oats are a widely grown crop which provide important nourishment both as human food and as livestock feed in many countries. The oat plant is a prominent member of the cereal grass family and bears the botanical name *Gramineae Avena sativa*. The treatise entitled "L'Avoine" by the Frenchmen Denaiffe and Sirodot (1902) seems to be the most comprehensive publication on record devoted exclusively to oats. In the introductory part of their book of more than 800 pages oats are named in almost a score of languages, representing countries where the crop had importance at the beginning of the twentieth century. The respective names follow:

Allemand—Hafer; Anglais—Oat; Breton—Kerch; Danois—Havre; Espagnol—Avena; Flamand—Haver; Hollandais—Haver; Hongrois—Zab; Italien—Vena; Japonais—Enbaku; Latin—Avena; Norwegian—Havre; Polonais—Owies; Portugais—Veia; Roumain—Ovesul; Russe—Ovesu; Serbe—Ovas; Suedois—Hafre; Turc—Ionlaf.

Records indicate that the oat plant was originally observed more than forty centuries ago and that during the Bronze Age, 1500–500 B.C., it was first developed as a domestic crop. By the beginning of the seventeenth century the crop was extensively established in Western Europe. At that time, 1602, oat seeds are said to have been transported across the Atlantic Ocean to be planted for the first time in North America—in the New England States. Their cultivation in this country expanded to the point that George Washington is said to have sown on his plantation at Mt. Vernon, Virginia, in 1787 almost 400 acres of oats. From those early American colonial days oats have

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become progressively more important as a feed and food crop in the United States, being grown particularly in the northern States and consumed in all sections.

## Culture of Oats

Two generations ago the American production of oats occurred mainly in the States of Ohio, Pennsylvania and New York. Gradually, with the settlement of newer areas, the culture of oats moved westward and northward until the chief oats-producing section now coincides rather closely with the so-called north-central "corn belt." One important difference, however, is that the oats area extends farther north than does that of corn. In terms of millions of bushels (32 pounds per bushel) produced in 1935, the foremost production figures for individual States were: Iowa 205, Minnesota 181, Illinois 107, and Wisconsin 85.

Oats fit well into the rotation scheme now prevalent in the diversified farming areas, being often grown between corn and wheat or between corn and grass or legumes.

Table I shows data taken from the Statistical Supplement of the latest United States Department of Agriculture Yearbook of Agriculture (1936a). According to the figures, oat production reached its peak about the year 1915 with a volume of 1,435,270,000 bushels.

TABLE I

OATS ACREAGE, PRODUCTION, AND VALUES IN U. S. A. AT DECENNIAL INTERVALS

| Year | Acreage harvested (1000 acres) | Production (1000 bu.) | Farm value (1000 dollars) |
|---|---|---|---|
| 1875 | 13,616 | 364,967 | 134,088 |
| 1885 | 23,351 | 674,151 | 187,860 |
| 1895 | 30,905 | 924,858 | 178,767 |
| 1905 | 33,426 | 1,104,395 | 317,843 |
| 1915 | 38,802 | 1,435,270 | 550,065 |
| 1925 | 44,240 | 1,410,336 | 547,231 |
| 1935 | 39,714 | 1,195,435 | 333,960 |

In terms of domestic tonnage produced in 1935 the three leading cereal grains named in order of decreasing volume are corn (maize), oats, and wheat. If one arbitrarily assigns corn tonnage the value of 100, then the comparative figure for oats is 35, and for wheat it is 33. The severe drouth which has prevailed through the grain belt during the last six or seven years is responsible in part for the lower 1935 production of corn, oats, and wheat.

## Botany of Oats

The botanical classification of the oat plant, *Gramineae Avena sativa*, is summarized very thoroughly by Stanton (1936) of the United

States Bureau of Plant Industry, who has kindly permitted reproduction of his excellent photograph of the heads of several stalks of oat plants (Figure 1).

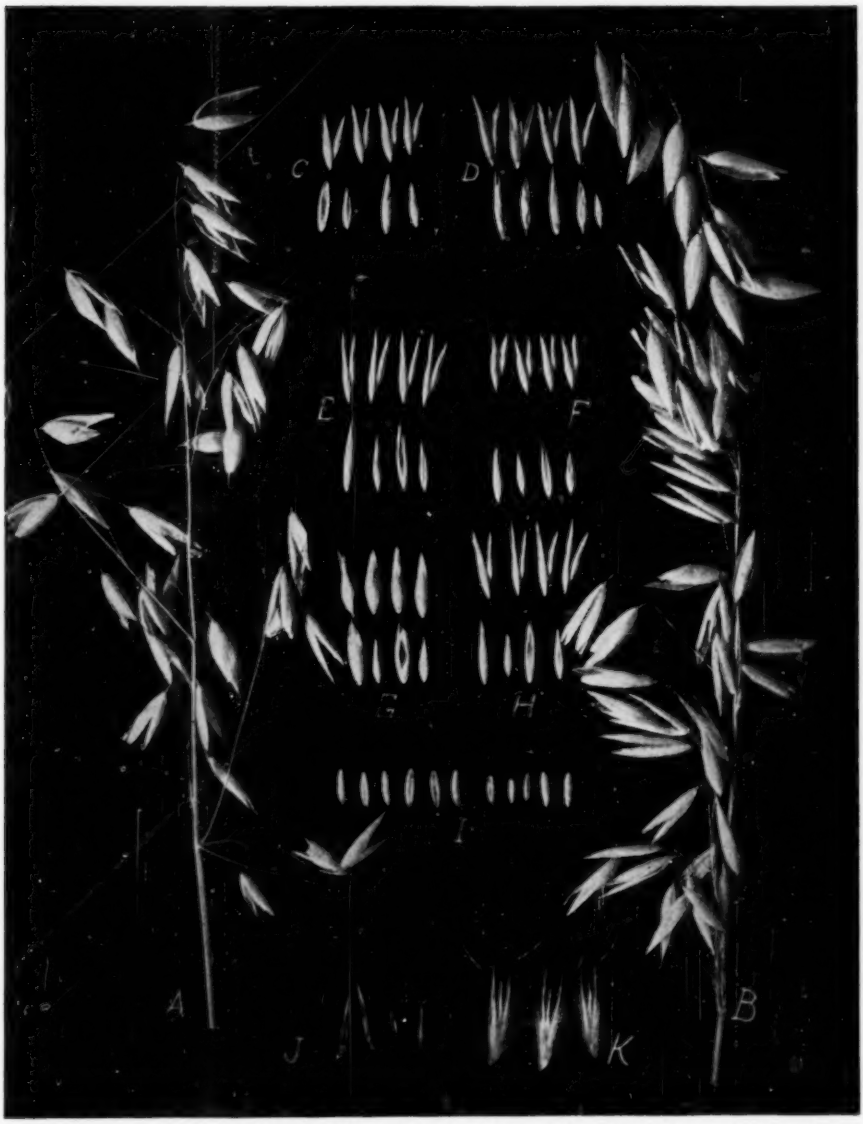



Figure 1. Panicles, spikelets, and florets of oats: (*A*) Equilateral (spreading or open) panicle; (*B*) unilateral (side or horse-mane) panicle; (*C*) common oats; (*D*) red oats; (*E*) long grains; (*F*) short grains; (*G*) plump grains; (*H*) slender grains; (*I*) hull-less or naked oats; (*J*) common wild oats (*Avena fatua L.*), and (*K*) wild red oats (*A. sterilis L.*).

In regard to the botany of oats, Stanton wrote as follows:

"Under average conditions, the oat plant produces from three to five hollow stems, or culms, varying from one-eighth to one-fourth inch in diameter and from 2 to 5 feet in height. The roots are small, numerous, and fibrous, and penetrate the soil to a depth of several feet. The leaves average about 10 inches in length and five-eighths of an inch in width. The panicles, or heads, are either spreading (equilateral or treelike) or one-sided (unilateral, horse-mane, or bannerlike). By far the greater number of cultivated varieties are of the type with spreading panicles. The grain is produced on small branches, in spikelets, varying in number from 20 to 150 per panicle. The number of florets or grains in each spikelet, except in the hull-less or naked oat, varies from two to three. The spikelet is loosely enclosed within the outer glumes (chaff). The kernels, except in the hull-less oat, are tightly enclosed within the lemmas or inner glumes and palea. The lemma or hull varies in color from white, yellow, gray, and red to black and may be awned or awnless. The kernel, or more properly the caryopsis, without its adhering glumes, is very slender, ranging from five to seven sixteenths of an inch in length and from one to two sixteenths of an inch in width. The kernel constitutes about 65 to 75 percent of the total weight of the whole grain."

## Histology of the Oat Seed

Microscopic study of many kinds of seeds has been thorough and exhaustive. Drawings of the detailed interior and exterior structure of kernels of maize and wheat have been published in both lateral and longitudinal sections through different parts of the kernels, appearing as early as fifty years ago. One of the finest old works on detailed structure of the cereal grain seeds is the German text "Landwirtschaftliche Samenkunde I, II," by Harz (1885). That book, however, fails to show the structure of the oat kernel in longitudinal section through the embryo. Avery (1930) portrayed particularly the very highly magnified oat embryo. For those interested especially in the comparative structure of maize, oats, and wheat kernels Avery's article should prove extremely useful.

Reference to the extensive works of the contemporary authors Andrew L. Winton and Kate Barber Winton (1932) disclosed that their well-known book, "Structure and Composition of Foods, Volume 1," did not describe the oat kernel in great detail. Contact was therefore established with the Wintons and since they could recall from the literature no thorough drawing of a longitudinal section showing all important parts of the oat kernel, A. L. Winton was commissioned to make that study. Because of its typical character, its growing popularity and its practical importance, the Iogold variety was selected for the microscopic studies. Development of Iogold oats was started at Iowa State College about 1906 from a single plant of the Kherson variety. Particularly favorable characteristics of Iogold oats are the stiffness of straw, high yield, and excellent resistance to parasitic stem rust. L. C. Burnett, who generously furnished materials for this histological study, has described Iogold oats thoroughly in a bulletin of the Iowa State College Experiment Station (1928).

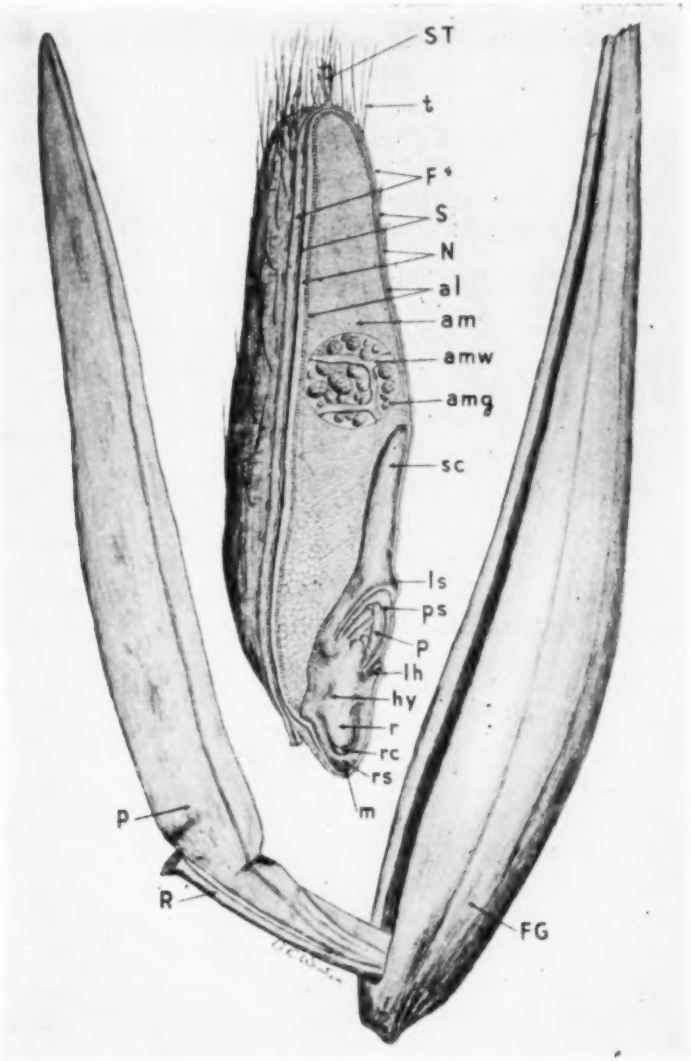



Figure 2. Oats (*Gramineae Avena sativa*). The drawing reproduced above shows a kernel of Iogold oats, lifted out of its enveloping chaff or hulls (flowering glumes and palet), partially sectioned longitudinally through the cleft and with a very restricted part of the endosperm further enlarged as an inset. The drawing was made by Andrew L. Winton, sometime State and Federal Chemist, co-author of "The Structure and Composition of Foods" in four volumes (John Wiley & Sons, Inc.), and other publications.

The scale of original drawing was 22 times natural size for all parts except the inset, which was 300 times natural size; as printed above, the scale is 11 times natural size for all parts except the inset, which is 150 times natural size.

Naming and lettering are done in accord with the internationally convenient policy of adhering as closely as possible to the Latin. Capital letters distinguish morphological parts such as fruit coat, seed coat, and others, while the smaller letters indicate layers of cells and their contents making up such parts.

*Parts of kernel or groat:* (*ST*) remnant of style, (*t*) trichomes or hairs, (*F*) fruit-coat or pericarp, (*S*) spermoderm or seed-coat, (*N*) hyaline layer or perisperm, (*al*) aleurone layer of endosperm, (*am*) amylaceous or starchy endosperm, (*sc*) scutellum or cotyledon of embryo (germ), (*ls*) ligule of scutellum, (*ps*) plumule sheath, (*p*) plumule or leaf bud, (*lh*) ligule of hypocotyl, (*hy*) hypocotyl or embryonic stem, (*r*) radicle or embryonic root, (*rc*) radicle cap, (*rs*) radicle sheath, (*m*) micropyle or opening through which the pollen entered.

*Inset: Aggregates of starch grains in starch cells.* (*amw*) starch cell walls; (*amg*) starch grains (numerous polygonal starch grains closely associated constitute the clusters of starch grains).

*Parts of chaff or hulls:* (*FG*) flowering glume, (*P*) palet, (*R*) stem of upper kernel.

Hundreds of kernels and sections of kernels were observed by A. L. Winton and from them the pencil drawing shown in Figure 2 was made. Winton (1936) wrote in regard to the drawing:

"The two novel features which the picture brings out are the long scutellum and the transition from transversely elongated starch cells above to the isodiametric cells below."

The explanation, legend and lettering given in Figure 2 are also in accord with the designations by Winton.

## Official Grain Standards Regarding Oats

For the purposes of the United States Grain Standards Act the United States Department of Agriculture (1936) has described oats in part as follows:

*Oats* shall be any grain which consists of 80 percent or more of cultivated oats. Oats may contain not more than 10 percent of wild oats.

Oats are further classified according to color as follows:

Class I, White Oats; Class II, Red Oats; Class III, Gray Oats; Class IV, Black Oats; and Class V, Mixed Oats.

It is pointed out that "White Oats" shall include yellow oats. Other details of the oat standards concern themselves with soundness, brightness, and degree of infestation of the grain.

Official and non-official definitions of oats and their milled products follow after the section on milling.

## Milling of Oats

Oat-milling procedure is especially adjusted to the structure of the oat kernels and of the chaff (or hulls) which closely envelopes each individual kernel through the threshing and well into the milling at the processing plant. A mill equipped effectively to process oats could not satisfactorily mill wheat and the converse is equally true.

Oats are purchased from country or terminal grain elevators subject to inspection to assure that only oats of suitable high grade enter the mill. Soundness of kernels and freedom from heat damage, foreign odors, wild onion seed, smut, must, and molds are of vital importance. At the mill oats are placed in large elevators. Moisture content of more than 13% is likely to cause heating and discoloration damage. To guard against that, moving and aeration of grain to lower its temperature can be accomplished when necessary.

The oat mill flow-sheet, as shown in Figure 3, indicates the most important steps in the processing of oats. Sunderman (1931) has also discussed oat-milling methods. The initial step—cleaning of the oats—removes foreign materials such as metallic pieces, dust, chaff, weed

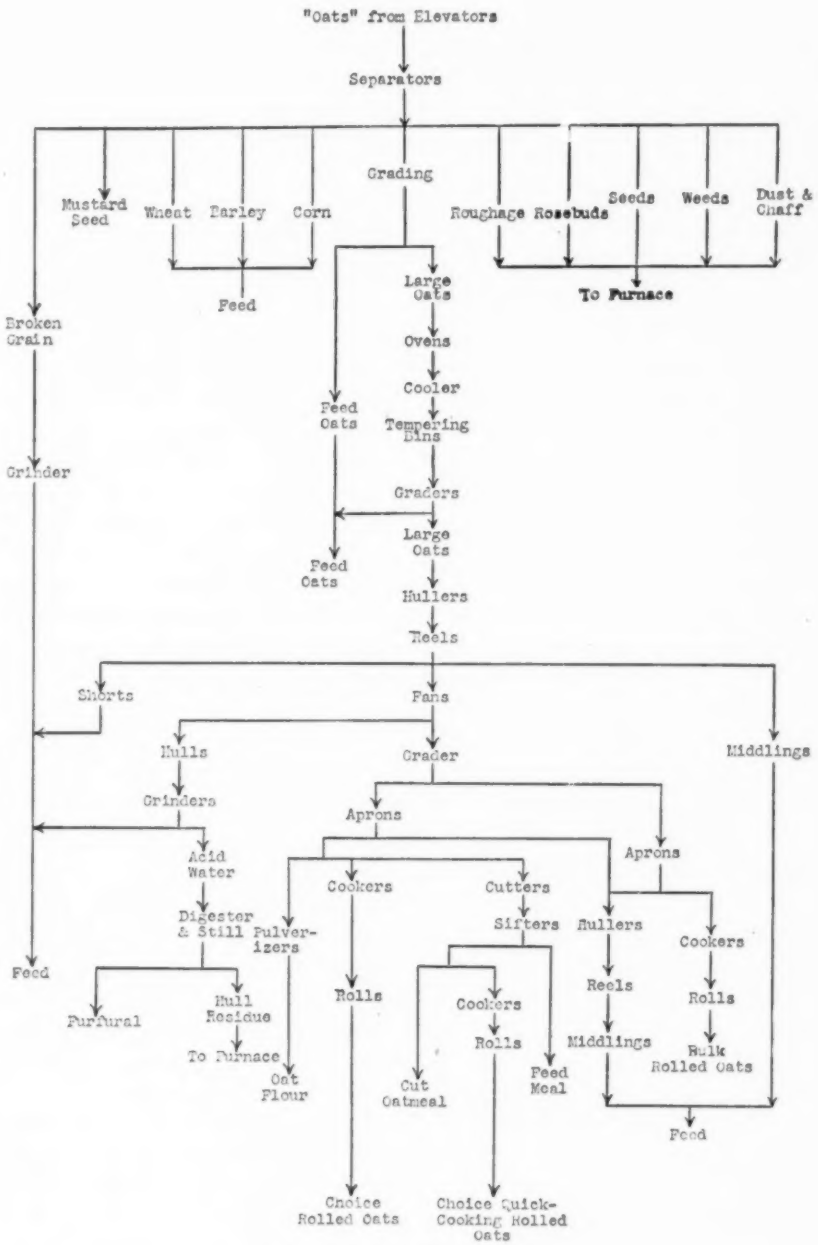



Figure 3. Oat mill flow-sheet.

seeds, wheat, corn and barley, as well as oats which are either small, light or double. Some thirty different machines, or separators, are employed to remove these foreign materials. The separators serve specific purposes and are of the following general types: air, disc, grading reel, grading separator, apron, cylindrical, centrifugal, mustard seed, and magnetic.

When the cleaning is completed the oats are conveyed to the so-called drying house where the moisture content is reduced from about 10 or 12% to about 6%. Desiccation is accomplished in a vertical stack of large open steel pans, the bottoms of which are steam jacketed. Oats fed to the center of the top pan are slowly stirred outward by a sweeping paddle which moves them toward the edge of the pan where they drop into a chute and are directed to the middle of the next lower pan. This procedure is repeated from 8 to 12 times. In the trade various names such as roasting, toasting, dry cooking, or cooking are sometimes applied to that treatment. The roasting stage is of approximately one-hour duration throughout which a temperature of substantially 180° F. is maintained in the oats. This heating makes the hulls more brittle, thus facilitating their subsequent removal, and in addition the slow roasting develops a certain desirable flavor in the groat which persists into the final product.

From the bottom of the roasting stack the oats which are still tightly enveloped individually by the hulls are taken through an air cooler. The oats are then ready for the oatmeal mill proper in which the chief steps are: separation of oat grains into grades closely identical as to diameter and length; hulling; separation of the hulls and the unhulled oats from the groats; steaming the groats preparatory to rolling; rolling the groats into flakes; and packaging.

In the precise separations of oats according to size the largest kernels usually constitute a majority and are designated "large oats," while other fractions are called "slim oats," "long slims," "No. 1 stub," "No. 2 stub," and "No. 3 stub," respectively. Each of these streams is sent separately to the hulling stones, the "large" oats going to make the choicest grade of large flakes, while the less choice or smaller oats may be converted into so-called bulk rolled oats.

One of the most fascinating operations in an oat mill is the hulling of the grains, the tearing of the hulls or glumes and palet from the kernels or groats. To remove the hulls the oats are passed between two large circular stones about three feet in diameter, mounted horizontally one immediately over the other. The upper surface of the lower stone is almost flat while the lower surface of the upper stone is slightly conical. The upper stone rotates rapidly. The lower stone is stationary. Oats under flow of gravity pass through an opening in

the center of the upper stone and are gradually forced to the outer edge of the pair of stones. The stones are precisely adjusted so that the intervening space is just slightly greater than the length of the groats in the grains to be hulled. En route between the center and outer edge of the stones the grains are turned on end. Hulls are shattered and torn from the groat. The yield of unbroken groats is determined by the skill and perfection with which the stones are "dressed" and adjusted. Formerly the hullers were actually of stone but now they are of manufactured silicon carbide composition.

From the hulling stones the mixture of loosened hulls, whole oat groats, and bits of floury pieces broken from the groat are directed through sieves, reels and purifiers which make sharp separations of the various fractions. The oat groats obtained at this point may be processed by any one of a large variety of methods each of which would give rise to a distinctive food, feed, or industrial product. While a small amount of whole-oat groats is used in that form for special feeding purposes, most of the groats are transformed to other products. Of greatest prominence among products made from oat groats are the flakes or rolled oats. Whole groats destined for the larger ("standard") flake are steamed directly with live steam at atmospheric pressure for a few minutes preceding the rollers. This softens the kernels so that pulverization is kept to a minimum when the groat is flattened as it passes between precisely adjusted heavy steel rolls.

If, in contrast, the groats are to be made into the so-called quick-cooking or smaller type flakes, it is first necessary to steel-cut the groats into pieces by means of rotary type granulators. Several distinct degrees of granulation are thus obtained which are often classified simply as A (finest), B (medium), and C (coarsest) steel-cut or "cut oats." Steel-cut or "Scotch Oats" were at one time the chief form in which oats were consumed as a human food, being displaced later by rolled oats. In the medium steel-cut oats each granule represents about one-third of a groat. That is the size usually employed for making the quick-cooking rolled oats, one granule making one flake. One entire groat is required to make one flake of the larger "standard" or slower cooking type of rolled oats. The rolling of the medium steel-cut oats is accomplished by technique identical with that used for the larger flakes except that the rolls are set closer together to make thinner flakes. Heat treatment of the two kinds of flakes is similar.

Milling percentage of rolled oats varies widely. It is dependent especially on quality of the grain. The heavier, plumper, dryer, and cleaner oats give the best milling yield. The skill of the millwrights in dressing and adjusting the hulling stones, as well as in operating numerous other machines, has great influence on the yield of rolled oats.

With medium good quality No. 2 White Oats as they are usually received at the mill, about 13.5 bushels or 432 pounds are required to produce a barrel of good quality rolled oats. A "barrel" of rolled oats or oat groats is 180 pounds. In contrast, a "barrel" of ground oatmeal or steel-cut oats is 196 pounds.

### Definitions of Oats and Certain of Their Milled Products

The only official definitions of oat products are those by the Association of American Feed Control Officials, Inc. (1937). Their definitions regarding oat products are reproduced in full as follows:

"*Oat Hulls* is the product consisting of the outer covering of the oat.

"*Oat Middlings* is the product consisting of the floury portions of the oat groat obtained in the milling of rolled oats.

"*Oat Shorts* is the product consisting of the covering of the oat grain lying immediately inside the hull, being a fuzzy material carrying with it considerable portions of the fine floury part of the groat obtained in the milling of rolled oats.

"*Oat Chop, Ground Oats, Pulverized Oats, Crushed Oats, or Crimped Oats* consists of the entire product made by chopping, cutting, grinding, crushing, or crimping whole oats.

"*Oat Groats* are the kernels produced from cleaned and dried oats in the process of manufacturing oat meal.

"*Hulled Oats or Undried Oat Groats* are the kernels produced from the undried grain in the process of hulling oats.

"*Oat Meal or Ground Oat Groats* is the product produced by cutting, cracking or grinding oat groats.

"*Rolled Oat Groats or Rolled Oats* is the product obtained in the process of rolling oat groats.

"*Clipped Oat By-Products* is the by-product obtained in the manufacture of clipped oats. It may contain the light chaffy material broken from the end of the hulls, empty hulls, light immature oats and dust. It must not contain an excessive amount of oat hulls.

"*Oat Mill Feed* (Oat Hulls, Oat Shorts and Oat Middlings) is the entire by-product produced in the manufacture of oat groats and consists of oat hulls, oat shorts and oat middlings. If used in a mixed feed, it shall be called Oat Mill Feed (Oat Hulls, Oat Shorts and Oat Middlings)."

In the absence of official definitions covering still other materials of importance, it seems advisable to describe them non-officially as follows:

*Oats*—often refers to cereal grass plants of the *Gramineae Avena sativa* classification. The word may also mean, according to equally common usage, the grains of oats with their hulls still intact. It is in this form that the grains leave the thresher or "combine" harvester and continue through several of the early stages of processing at the oat mill.

*Ground Oats*—the grains including hulls and groats in their natural proportions, ground to a mealy character generally in an attrition mill.

*Ground Oat Meal*—oat groats ground to a mealy consistency in an attrition mill.

*Steel-cut Oats, Cut Oats, or Scotch Oats*—oat groats cut by special steel rolls into granular pieces varying in size according to arbitrary

trade specifications dependent on size of granules. Three sizes are commonly designated, namely: A—finest, B—medium, and C—coarsest.

*Rolled Oats*—oat kernels (groats), or steel-cut (cut) oats including endosperm, bran and germ which have been pressed between two rolls in such a manner as to produce flattened discs. There are substantially two well-known types of rolled oats. "Regular" or "standard" rolled oats are the flakes produced by rolling whole oat groats, in which case one whole groat produces one large flake. "Quick-cooking" rolled oats are the smaller, thinner flakes produced by rolling steel-cut oats. Usually the medium-sized cut oats are used to make quick-cooking flakes, which are both thinner and much smaller in diameter than are "standard" rolled oats.

*Oat Flour*—finely ground and bolted flour made from oat groats. A variety of oat flours is produced. Depending on the use for which they are designed, the range of flours varies chiefly in texture, color, and fiber content.

*Oatmeal*—a term which has a wide variety of meanings. Literally, it means the meal of oats. It is also used less specifically to designate steel-cut or rolled oats. The term may also be used to indicate the porridge made from any of these three general forms of oat foods. However wide and non-specific the term may have become, the circumstances of its use generally give automatic intimation as to the precise product concerned.

### Composition of Oats and Certain Oat Products

Figures typical of the chemical composition of oat grains, oat kernels (groats), and of rolled oats are reproduced in Table II, from Sherman's book on *Food Products* (1933).

TABLE II

ANALYSES OF OATS AND OAT PRODUCTS

| | Oat grains, kernels with hulls | Oat kernels, without hulls | Rolled oats |
|---|---|---|---|
| | % | % | % |
| Moisture | 10.06 | 6.93 | 7.7 |
| Protein | 12.15 | 14.31 | 16.7 |
| Fat | 4.33 | 8.14 | 7.3 |
| Fiber | 12.07 | 1.38 | 1.3 |
| Carbohydrates, other than fiber | 57.93 | 67.09 | 66.2 |
| Ash | 3.46 | 2.15 | 2.1 |

Further data, concerning particularly the approximate mineral content of rolled oats as reported by Miner (1926, 1933), are indicated in Table III.

TABLE III

APPROXIMATE MINERAL CONTENT OF ROLLED OATS

| | | | |
|---|---|---|---|
| Calcium, expressed as CaO | 0.083% | Chlorine, expressed as Cl | 0.090% |
| Phosphorus, expressed as $P_2O_5$ | 0.920% | Magnesium, expressed as MgO | 0.260% |
| Iron, expressed as $Fe_2O_3$ | 0.0043% | Potassium, expressed as $K_2O$ | 0.200% |
| Copper, expressed as CuO | 0.000423% | Silicon, expressed as $SiO_2$ | 0.049% |
| Manganese, expressed as $Mn_3O_4$ | 0.0098% | Sodium, expressed as $Na_2O$ | 0.140% |
| Aluminum, expressed as $Al_2O_3$ | 0.0123% | Sulphur, expressed as S | 0.225% |
| Bromine, expressed as Br | 0.00039% | | |

## Oat Foods

According to citations by Thornton (1933) the entire supply of oat foods for human consumption in the United States up to about the year 1850 was imported from Scotland and Canada. Oatmeal then was sold in drug stores as a remedial agent. Starting about the middle of the nineteenth century the milling of oat foods was commenced in Ravenna, Ohio. Since that day the increase of oat foods manufacture has grown with remarkable speed.

For a period of substantially seventy or eighty years the "bowl of oatmeal" has been used with traditional regularity to designate a serving portion of America's most widely consumed porridge. In some sections of the country the words "porridge" and "oatmeal" are considered synonymous and are used interchangeably. The fact that oatmeal has been marketed in four different physical forms makes little difference to many consumers who have preferred to use the word oatmeal in its broad sense. In the earliest years of American production of oat foods the groats were ground into a meal; at a later period steel-cut oats rose to greater popularity. Steel-cut oats were followed by the large flakes and latterly they in turn have been partially supplanted by the smaller or quick-cooking type of flakes. Today the chief food products of an oat mill are the two kinds of flakes or rolled oats, and by far the larger proportion of these are packed in fiber packages in contrast with the barreled, or sacked, oatmeal of earlier periods. The smaller flakes continue to win the favor of many consumers because they are more quickly cooked. The starch of the smaller flakes is more rapidly swollen and ruptured because the thinness permits more ready penetration by the hot water and steam.

The composition of oat foods of all shapes or forms is substantially similar. Therefore, one set of figures serves satisfactorily for oat groats, steel-cut oats, ground oats and most oat flours. Tables II and III show these data for oats and oat products. Table IV indicates composition of oats and their chief milled products in comparison with corresponding data for five other cereal grains.

TABLE IV

APPROXIMATE COMPOSITION OF CEREAL GRAINS AND OF CERTAIN CEREAL FOODS

| Grain or product | Moisture | Protein | Fat | Crude fiber | Carbohydrate[1] | Ash | Calories | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Per 100 grams | Per ounce |
| | % | % | % | % | % | % | | |
| *Barley* (*Gramineae Hordeum sativum*—family, genus, species) | | | | | | | | |
| Entire grains with hulls | 11.9 | 10.5 | 2.2 | 3.8 | 72.8 | 2.6 | 350 | 100 |
| Pearled barley | 10.8 | 8.5 | 1.1 | 0.5 | 77.8 | 1.3 | 355 | 101 |
| *Corn* (*Gramineae Zea mays*) | | | | | | | | |
| Entire grains or kernels | 10.7 | 10.0 | 4.3 | 1.7 | 71.8 | 1.5 | 366 | 104 |
| Degerminated corn meal | 11.0 | 7.8 | 1.3 | 0.8 | 78.5 | 0.6 | 357 | 101 |
| *Oats* (*Gramineae Avena sativa*) | | | | | | | | |
| Entire grains with hulls | 10.1 | 12.2 | 4.3 | 12.1 | 57.8 | 3.5 | 319 | 90 |
| Entire kernels without hulls (groats), oatmeal (ground), or rolled oats | 8.7 | 16.0 | 5.6 | 1.2 | 66.7 | 1.9 | 380 | 108 |
| *Rice* (*Gramineae Oryza sativa*) | | | | | | | | |
| Entire grains with hulls, "Rough rice" | 11.7 | 8.1 | 1.8 | 8.9 | 64.3 | 5.2 | 305 | 86 |
| Brown rice (entire kernels, without hulls) | 12.1 | 9.1 | 2.0 | 1.1 | 74.6 | 1.1 | 353 | 101 |
| Polished rice | 11.5 | 7.0 | 0.6 | 0.8 | 79.3 | 0.8 | 352 | 100 |
| *Rye* (*Gramineae Secale cereale*) | | | | | | | | |
| Entire kernels without hulls | 9.4 | 11.8 | 1.8 | 1.8 | 73.2 | 2.0 | 356 | 101 |
| Rye flour | 11.1 | 7.9 | 1.1 | 0.4 | 78.7 | 0.8 | 356 | 101 |
| *Wheat* (*Gramineae Triticum sativum*) | | | | | | | | |
| Entire kernels without hulls | 10.3 | 12.4 | 2.2 | 2.2 | 71.0 | 1.9 | 353 | 101 |
| Patent flour (white) | 11.0 | 11.0 | 1.3 | 0.2 | 76.1 | 0.4 | 360 | 102 |

[1] Calculated by difference—crude fiber excluded.

As a source of vitamins oats have a trace of vitamin A, they are a very good source of vitamin $B_1$, they are devoid of vitamins C and D, their content of riboflavin and other parts of the vitamin G ($B_2$) complex is uncertain, and they are considered to contain appreciable vitamin E. Vitamin $B_1$ assays have been made in the laboratory of one of the authors on successive stages of oats from the grain elevator to rolled oats porridge. It was found that roasting for flavor development did not impair the $B_1$ content, that there was a slight loss at the flaking stage, and a further slight loss on cooking to a porridge. The figures in terms of International units of vitamin $B_1$ per ounce (on the air-dry basis) are respectively: raw oats 62, "toasted" oats 62, oat groats 62, rolled oats 54, and rolled oats porridge or oatmeal 47. For comparison it can be indicated that the vitamin $B_1$ potency of raw, unheated, unmilled whole wheat has been found in simultaneous tests

to assay approximately 40 International units per ounce. Thus it is evident that rolled oats porridge is a very good source of vitamin $B_1$.

The greatest consumption of oat foods occurs in the so-called "breakfast food" group. As used for that purpose for children and adults alike the cooking methods employed vary widely from family to family. In general, one ounce of rolled oats produces about four ounces of porridge. That amount constitutes a typical serving portion which is usually eaten with added milk or cream either with or without a bit of sugar. Rolled oats are also used in numerous other culinary capacities, as for example cookies, breads, and puddings.

National production figures for oat breakfast foods provide the closest indices available concerning the annual consumption of these foods. Exportation and importation of "breakfast foods" from this country are so small as to be of little significance. Latest Federal statistics on production compiled by the United States Department of Commerce (1937) are indicated in Table V. These figures concern the production year 1935.

TABLE V

CEREAL "BREAKFAST FOOD" PRODUCTION IN THE UNITED STATES DURING 1935

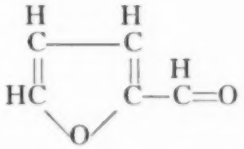
| | |
|---|---|
| Breakfast foods: | |
| Total pounds | 1,151,546,585 |
| Total value | $99,344,148 |
| Made from wheat: | |
| Pounds | 396,067,131 |
| Value | $45,537,950 |
| Made from oats: | |
| Pounds | 528,934,528 |
| Value | $26,386,380 |
| Made from corn: | |
| Pounds | 184,348,608 |
| Value | $18,792,603 |
| Made from other grains and from mixed grains: | |
| Pounds | 42,196,318 |
| Value | $8,627,215 |

The data show that oat foods, substantially all of which are rolled oats, constituted more than 46% of all breakfast foods produced. It is interesting, especially in view of the more recent findings and opinions in regard to nutrition and dietetics, that all of the oat foods are whole-grain foods containing the bran and germ as well as the endosperm portions of the kernels. Corn breakfast foods are almost entirely "refined" in the sense that the bran and germ have been removed. Of the wheat products it may be estimated that approximately one-third of the wheat breakfast foods are whole-grain foods. On the basis of these figures one can then indicate that the American public consumes its whole-grain foods in approximately the following quantities per annum:

| | | |
|---|---|---|
| Oat breakfast foods | 528,934,528 | pounds |
| Wheat breakfast foods | 132,022,377[4] | pounds |
| Graham and whole-wheat flour | 307,431,684[5] | pounds |

Other uses beyond the fields of human food and livestock feed have been found for various oat products. Among these are the chemical compound furfural and its derivatives which are made from oat hulls, oat flour as an agent to retard rancidity development in foods, and oat flour as either an ingredient in soaps or as detergent agent used in lieu of soaps. Since circumstances do not permit a detailed review of each of these uses here, it will perhaps suffice to describe those uses briefly and provide references to more exhaustive publications covering the respective phases of oats.

Furfural is a liquid aldehyde, light amber in color, having an odor which is not unpleasant. Furfural is made by destructive distillation of oat hulls in the presence of acid and steam under regulated conditions. In that way the pentosans are cleaved first to pentoses and then dehydrated to furfural. The structural formula of furfural follows:



The anatomical differences between grains of oats and wheat, particularly the fact that the oat hulls are not removed in threshing, is basically responsible for the furfural industry's close connection with oats. The corresponding hulls of wheat, in contrast, readily yield to separation from the kernels when threshed. Because of its aldehyde structure which condenses successfully with phenols, ketones, or amines to form resins, many of the widely used plastics are now made in part from furfural. Its solvent properties have commended it to use in paint removers, lacquer solvents, as selective solvents for purifying petroleum lubricating oils and for numerous other purposes. A review of furfural and other furans together with a considerable bibliography was published by Peters (1936).

Oat meal (or flour) soaps and bath "packs" have been used for years, both for ordinary cleansing and beautifying purposes as well as for treatment of dermatitis and eczema. As a constituent of certain soaps, the oat meal adds pleasant and effective detergent qualities. Latterly there has been produced a perfumed oat flour which is used both for therapeutic and for more widespread cosmetic applications either in bath water or directly to the skin as a powder or cream.

[4] One-third of wheat volume figure shown in Table V.
[5] Taken from the 1935 production figure given on page 5 of the previously cited Bulletin by the United States Department of Commerce.

Oat flour has the effect of retarding rancidity in certain foods, particularly those high in fat content, and is gaining increased usage therefore particularly in the field of human food and animal feed where higher potency chemical antioxidants are objectionable because of toxicity. Recent progress in this further expansion of uses for oat flour is summarized in a review by Peters and Musher (1937).

## Summary

Oats, *Gramineae Avena sativa*, is an important human food and livestock feed crop of this country. In terms of tonnage produced in 1935 it ranks immediately ahead of wheat and is second only to corn in the cereal group. This article includes original publication of a drawing by A. L. Winton showing detailed microscopic structure of the seed (or kernel) of Iogold oats in partial longitudinal section. The method of milling oats, descriptions and definitions of related products, composition and uses of the oat foods, feed materials, and industrial products are discussed.

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# THE NATURE OF THE INCREASE IN AMYLASE ACTIVITY OF GERMINATING BARLEY [1]

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(Read at the Annual Meeting, May 1937)

The increase in amylase activity which occurs upon the germination of barley and other cereal grains was attributed by early investigators to the actual synthesis of enzymes, as indicated by Lüers (1936) in his review of the earlier investigations. The results of more recent researches show that this concept is probably erroneous.

Ugrümow (1935) found that both α- and β-amylases were present in the earlier stages of development of wheat kernels. The α-amylase activity disappeared entirely as the grain passed through the "milk-ripe" stage and did not appear again until the third day of germination. The β-amylase activity of the maturing kernels decreased slightly until it reached a minimum at full maturity. The increase in β-amylase activity began at the end of the steeping period in the malting operation and continued until an increase of approximately 100% was observed by the second week of germination.

Chrzaszcz and Janicki (1936) have shown that this sequence of changes in amylase activity during ripening and subsequent germination is characteristic also for barley and other cereals. It was observed that the dextrinizing power of ripening barley disappeared completely during the "milk" stage and did not appear again until the fourth or fifth day of germination.

There have been several attempts in recent years to explain the increase in diastatic activity observed on germination of barley. Waldschmidt-Leitz and Purr (1931) presented evidence to show that this increase may be caused by the formation on germination of barley of an amylase activator which they have designated as "amylokinase." The activator was separated from the amylases of green malt extracts by adsorbing the amylases from solution with Tonerde Cγ at pH 5.0. When this concentrated amylase-free activator solution was added to an extract of ungerminated barley, they observed a 200% increase in maltose production.

[1] Paper No. 1549, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Claude H. Hills to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

The "amylokinase" was non-diffusable through animal membranes and was not entirely destroyed by heating to 100° C. for 30 minutes. Thus it may be of the nature of a protein degradation product. These authors concluded that the "amylokinase" activated both α- and β-malt amylases, but had no effect on pancreatic amylase.

Lüers and Rümmler (1935) were unable to confirm Waldschmidt-Leitz and Purr's results. A similar inability to confirm the "amylokinase" hypothesis was reported by Weidenhagen (1933) who stated further that the activator had no action on pure amylases but protected against inhibitory substances in crude extracts.

Chrzaszcz and Janicki (1933b) explained the changes in amylase activity on germination of barley by postulating the presence of an amylase inhibitor, "sisto-amylase." The increase in amylase activity on germination was attributed to the ability of the protein degradation products to counteract the effect of the "sisto-amylase" and thus restore the full activity of the amylases. The action of this inhibitor could be counteracted by certain "eleuto-substances" such as peptone or NaCl. Chrzaszcz and Janicki (1936) attributed the decrease in amylase activity during the ripening of barley to an accumulation of "sisto-amylase."

The concentration of "sisto-amylase" in barley or malt was determined by measuring the increase in amylase activity upon the addition of peptone to the raw extracts. The fact that "sisto-amylase" occurred in small amounts in ungerminated barley and increased rapidly on germination seems to be inconsistent with the conclusion that the decrease in amylase activity on ripening of cereals was due to an accumulation of the inhibitor.

The studies of Oparin, Manskaja and Magaram (1933) led to a different interpretation of the effect of peptone on barley malt extracts. They observed that egg albumin increased the thermo-lability of amylase in solution. If the added egg albumin was denatured by physical or chemical means it adsorbed part of the soluble amylase on the insoluble protein precipitate. Peptone partially protected malt amylases against this type of inactivation.

Oparin, Manskaja, and Glasunow (1934) concluded that a tannic acid precipitate of egg albumin, when added to a barley malt extract, behaved like the "sisto-amylase" described by Chrzaszcz and Janicki. They stated the belief that peptone peptized the amylase adsorbed on insoluble protein.

Ford and Guthrie (1908) first demonstrated that the digestion of barley extracts with papain increased amylase activity. More recently Myrbäck and Myrbäck (1933) employed this technique to de-

termine the "free" and "bound" amylase content of barley. By digesting a barley meal suspension with papain they observed a 200% to 300% increase in amylase activity. Thus they explained the increase in $\beta$-amylase activity on germination of barley as due to the proteolytic release of insoluble amylase bound to protein.

## Experimental

The existence and possible nature of amylokinase was investigated by repeating some of the original experiments of Waldschmidt-Leitz and Purr (1931, p. 124). They removed the amylases from a green malt by adsorbing with Tonerde C$\gamma$. When 8 c.c. of this amylase-free activator solution were added to .5 c.c. of barley extract they observed that the maltose production increased from 37.7 mg. to 120 mg.

The green malt used by us was prepared by steeping a 300 g. portion of barley in cold running water at 12.5° C. for 52 hours and then germinating in a small laboratory malting cabinet for 6 days at 16° C. The malted barley was frozen, ground through a food-chopper, and stored at —20° C.

*Green malt extract:* A green malt extract was prepared by extracting 2.5 g. (dry basis) of frozen malt with 50 c.c. of water, 3 hours at 21° C. The mixture was centrifuged and the extract decanted.

*Amylokinase solution:* To 25 c.c. of extract prepared from frozen green malt there were added 5 c.c. of 1.0 N acetate buffer (pH — 5.0). This extract was treated with four successive additions of 250 mg. (14.5 c.c.), 125 mg., 50 mg., and 50 mg. of Tonerde C$\gamma$. After each addition of Tonerde C$\gamma$ the mixture was allowed to stand for 5 minutes, and then centrifuged at half speed for exactly 5 minutes and the supernatant liquid decanted. After the last decantation the volume was made up to 50 c.c.

*Barley extract:* Ungerminated barley was ground through two series of corrugated rolls. To 2.5 g. of ground barley 50 c.c. of water were added, and the mixture extracted 3 hours at 21° C. and centrifuged. The extract was diluted 1:10 with water.

*Starch substrate:* A solution containing 25 c.c. of 2% soluble starch + 10 c.c. of 0.1 N citrate buffer (pH = 5.1) + enzyme additions + water to make 46 c.c. final volume, was digested 30 minutes at 37° C. Then 2 c.c. of 10% $H_2SO_4$ and 2 c.c. of 12% $Na_2WO_4$ were added. Maltose was determined in a 5 c.c. aliquot by Blish and Sandstedt's (1933) modification of the Hagedorn-Jensen method.

These results show no increase in maltose production due to the activator solution. The activator solution still contained a trace of

TABLE I

EXPERIMENTS DESIGNED TO DETECT AMYLOKINASE IN GREEN MALT EXTRACTS

| Number | Enzyme addition | Experiment I [1] | Experiment II |
|---|---|---|---|
| | | Mg. maltose per 50 c.c. | |
| 1 | 2 c.c. barley extract | 92.6 | 93.0 |
| 2 | 8 c.c. activator solution | 6.0 | 13.6 |
| 3 | Sum of No. 1 + No. 2 | 98.6 | 106.6 |
| 4 | 2 c.c. barley extract + 8 c.c. activator solution | 93.0 | 95.4 |
| 5 | Net increase due to activator (No. 4—No. 3) | −5.6 | −11.2 |
| 6 | % hydrolysis of starch substrate in No. 4 | 18.6% | 19.1% |
| 7 | 1 c.c. green malt extract diluted 1 : 10 | 100.0 | 106.0 |
| 8 | Calculated % removal of amylases from activator solution | 99.85% | 99.70% |

[1] Tonerde Cγ used in Experiment I was obtained from Waldschmidt-Leitz.

amylase activity although 99.7% to 99.85% of the amylases has been removed.

In a further effort to detect amylokinase in green malt extracts the above experiments were repeated with two minor changes in the procedure: fresh unfrozen green malt was used instead of frozen malt,

TABLE II

FURTHER EXPERIMENTS TO DETECT AMYLOKINASE IN GREEN MALT EXTRACTS

| Number | Enzyme addition | Number of experiment | | | | |
|---|---|---|---|---|---|---|
| | | IV | V | VI [1] | VII [1] | VIII [1] |
| | | Mg. maltose per 50 c.c. | | | | |
| 1 | 2 c.c. barley extract | 85 | 91 | 90 | 93 | 88 |
| 2 | 8 c.c. activator solution | 7 | 26 | 36 | 82 | 154 |
| 3 | Sum of No. 1 + No. 2 | 92 | 117 | 126 | 175 | 242 |
| 4 | 2 c.c. barley extract + 8 c.c. activator solution | 83 | 112 | 118 | 151 | 203 |
| 5 | Net increase due to activator (No. 4—No. 3) | −9 | −5 | −8 | −24 | −39 |
| 6 | % hydrolysis of starch in No. 4 | 16.6% | 22.4% | 23.6% | 30.2% | 40.6% |
| 7 | 2 c.c. green malt extract diluted 1 : 10 | 97 | 111 | 128 | 123 | 116 |
| 8 | % removal of amylases from activator solution | 99.65% | 98.83% | 94.40% | 86.7% | 73.5% |

[1] Two cubic centimeters of activator solution used in No. 2 and No. 4 instead of 8 c.c.

and smaller quantities of Tonerde Cγ were used since there was a possibility that the amylokinase had been adsorbed from solution also.

Accordingly, the experiments were repeated using fresh unfrozen green malt and a series of decreasing amounts of Tonerde Cγ, with the results shown in Table II.

The activator solution for each experiment was prepared by extracting the amylases from an extract of fresh unfrozen green malt by four successive additions of Tonerde Cγ.

| | |
|---|---|
| Experiment IV | 200 mg. (20 c.c.), 100 mg., 40 mg., and 20 mg. |
| Experiment V | 50 mg., 25 mg., 10 mg., and 5 mg. |
| Experiment VI | 40 mg., 20 mg., 8 mg., and 4 mg. |
| Experiment VII | 30 mg., 15 mg., 6 mg., and 3 mg. |
| Experiment VIII | 20 mg., 10 mg., and 4 mg. |

No increase in maltose production was observed due to the effect of the activator solution. The extent of extraction of amylases from solution ranged from nearly 100% to as low as 73.5%. These results agree with those of Lüers and Rümmler (1935) and of Weidenhagen (1933) who were unable to confirm Waldschmidt-Leitz and Purr's results.

### Effect of Papain and Peptone on α- and β-Amylase Activity

Myrbäck and Myrbäck (1933) have pointed out that part of the amylases of ungerminated barley are water-soluble and a considerable part of the active amylase is insoluble in water. They employed digestion with .5% papain for 24 hours to determine the "total" amylase activity of barley. The portion soluble in water was designated "free" amylase and the insoluble portion was called "bound" amylase.

Chrzaszcz and Janicki (1933) contend that the effect of papain in increasing amylase activity is not due to proteolytic release of bound amylase. Instead it is attributed to the ability of the protein degradation products to counteract the inhibitory effect of "sisto-amylase" present in such extracts. Accordingly, they employed extraction with 1% peptone to determine total amylase activity.

These two hypotheses have been tested by the experiments outlined in Tables III and IV. The α- and β-amylase activities were determined in the following manner:

*β-Amylase activity:* β-amylase activity was determined on barley and green malt extracts prepared by extracting 2.5 g. (dry basis) of barley or malt with 50 c.c. of water for 3 hours at 21° C. The extract obtained on centrifuging was diluted 1:10 with water. Then 2 c.c. of the diluted extract were added to 35 c.c. of 2% soluble starch containing 0.1 N acetate buffer (pH = 4.65). Additional 0.1 N acetate

buffer (pH = 4.65) was added to make a total volume of 46 c.c. and the mixture digested 1 hour at 30° C. At the end of 1 hour, 2 c.c. of 10% $H_2SO_4$ and 2 c.c. of 12% $Na_2WO_4$ were added and maltose determined on a 5 c.c. aliquot by the Blish-Sandstedt (1933) modification of the Hagedorn-Jensen method.

*α-amylase activity:* The modification of Wohlgemuth's (1908) method was to add graduated quantities of the enzyme extracts to 1 c.c. of 2% soluble starch solution buffered at pH 4.65 contained in test tubes. After addition of the enzyme extract, additional water was added to make a total volume of 6 c.c., the tubes were shaken, and the mixture digested 1 hour at 40° C. The tubes were then placed in an ice bath and 10 c.c. of ice-cold water and 1 drop of 0.1 N iodine solution were added. The end-point was taken as a deep violet color. The Wohlgemuth value, or α-amylase activity, expresses the number of cubic centimeters of 2% soluble starch dextrinized by 1 c.c. of the original enzyme solution in 1 hour at 40° C.

The effect of papain and peptone on the extraction of β-amylase from ungerminated barley is shown in Table III.

TABLE III

EXTRACTION OF TOTAL AMYLASE FROM BARLEY MEAL BY USE OF PAPAIN AND PEPTONE

| Number | Preparation of enzyme solution | Mg. maltose per 50 c.c. of digest |
|---|---|---|
| 1 | Extracted 3 hours with water | 129 |
| 2 | Residue from No. 1 washed with 50 c.c. of water | 18 |
| 3 | Residue from No. 2 digested 24 hours with .5% papain | 89 |
| | Total | 236 |
| 4 | Digested 24 hours with .5% papain (total amylase) | 248 |
| 5 | Extracted 3 hours with 1% peptone | 128 |
| 6 | 20 c.c. of solution No. 1 plus 50 mg. peptone | 129 |

Peptone did not increase the amylase activity of ungerminated barley when added to the extraction mixture (No. 5) nor when added to the clear extract (No. 6). These results are contrary to the observations of Chrzaszcz and Janicki (1933a, 1933b) who found that peptone increased β-amylase activity approximately 40–100%.

The results recorded in Table III confirm the findings of Myrbäck and Myrbäck (1936) who observed that papain digestion increased the amylase activity of ungerminated barley extracts approximately 100%, but peptone was without effect on β-amylase of barley.

It is apparent that total amylase thus determined (No. 4) is equivalent to the sum of the free amylase (No. 1) and the residual amylase (No. 2 and No. 3).

Table IV extends the investigation to both α- and β-amylases of green malt.

TABLE IV

EFFECT OF PAPAIN AND PEPTONE ON AMYLASE ACTIVITY OF GREEN MALT EXTRACTS

| | | β-amylase | α-amylase |
|---|---|---|---|
| Number | Preparation of enzyme solution | Mg. maltose per 50 c.c. | Wohlgemuth values |
| 1 | Green malt extracted 3 hours with water | 218 | 91 |
| 2 | Residue from No. 1 washed with 50 c.c. water | 23 | 6 |
| 3 | Residue from No. 2 digested 24 hours with .5% papain | 92 | 4 |
| | | Total 333 | 101 |
| 4 | Green malt digested 24 hours with .5% papain (total amylase) | 320 | 125 |
| 5 | 50 c.c. of solution No. 1 + 100 mg. peptone | 218 | 113 |
| | Total α-amylase (No. 5 + No. 2 + No. 3)..... | | 123 |
| 6 | Green malt extracted 3 hours with 1% peptone | 218 | 90 |

The increase in β-amylase activity due to papain extraction can be explained as the release of bound amylase from the residue of a water extraction. Peptone had no effect on the β-amylase activity of green malt (No. 5 and No. 6).

However, peptone had a noticeable activating effect on α-amylase. When peptone was added to a water extract of green malt, the α-amylase activity increased from 91 to 113, or about 24%. Less than 10% of the total α-amylase was released from the insoluble residue by papain (No. 3), which is quite in contrast to the large amount of residual β-amylase. Furthermore, total α-amylase is greater than the sum of the free and bound α-amylase. Much of the increase is due to the increase in activity of the soluble α-amylase.

To further test Chrzaszcz and Janicki's hypothesis concerning "sisto-amylase," peptone was added to a solution of purified α-amylase. The α-amylase preparation was secured from Olof Stamberg of the University of Minnesota, and was a very pure product as shown by its low saccharogenic activity.

A solution containing 1 mg. of α-amylase per cubic centimeter was capable of dextrinizing 14.1 c.c. of a 2% soluble starch solution in one hour at 40° C. However, when .5% peptone was added to the α-amylase solution its dextrinizing activity increased from 14.1 to 18.2 c.c. of 2% soluble starch dextrinized per hour. The peptone alone exhibited no dextrinogenic activity.

These results show that peptone increased the activity of solutions of purified α-amylase 29.1%, which is approximately the same increase as found for green malt extracts (24%). This increase may be explained in one of two ways: First, sisto-amylase, which is counteracted by peptone, is present in both green malt extracts and solutions of purified α-amylase, and to the same extent; or secondly, peptone may have an activating effect on α-amylase and thus affect green malt extracts and purified α-amylase to the same extent. This latter explanation seems to be the more plausible.

Oparin, Manskaja, and Magaram (1933) maintained that peptone increased amylase activity by its ability to peptize amylase adsorbed on insoluble protein particles. The effect of peptone on α-amylase may be explained by the observation that α-amylase is less soluble in water than β-amylase. Thus peptone may serve to increase the amount of α-amylase in solution.

## Summary

An investigation of the existence and possible nature of "amylokinase" failed to confirm the presence of "amylokinase" in green malt extracts of barley. Even when fresh unfrozen green malt was employed and the quantities of Tonerde Cγ adsorbent were varied over a considerable range, it was not possible to detect any amylase activator of the nature of "amylokinase."

Papain digestion increased the amylase activity of ungerminated barley approximately 100%. This increase in β-amylase activity was due to proteolytic release of β-amylase associated with water-insoluble material. It is concluded that papain digestion is a valid means of determining the total β-amylase activity of barley or barley malt.

Peptone did not affect the β-amylase activity of barley or malt. However, peptone increased the α-amylase activity of a green malt extract and of a solution of purified α-amylase 24% and 29% respectively.

The increase in α-amylase activity of green malt by papain extraction was not due to the release of bound amylase as in the instance of β-amylase, but was due largely to the increase in activity of α-amylase present in solution. The action of peptone in increasing the α-amylase activity of malt extracts is not clearly understood.

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# RELATION OF WATER ABSORPTION TO THE PHYSICAL PROPERTIES AND BAKING QUALITY OF FLOUR DOUGHS

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The term "absorption," when used in connection with the quantity of water added to flour in dough-making, is normally meant to signify that amount of water which in the judgment of the baker will produce a dough of optimum handling and baking quality for the particular flour used.

It is, however, common to find the terms "absorption" and "water content" being used indiscriminately, *e.g.*, "the flour was tested at the three absorptions (water contents) of 15, 16 and 17 gallons per sack," [1] and it would seem preferable to reserve the term "absorption" for the water content that produces a dough of optimum quality. The question then arises as to how this optimum condition is to be assessed.

The small commercial baker often uses the same quantity of water for all flours and makes but little attempt to judge the best conditions for each delivery, hence the necessity for good tolerance in bread-making flours. This condition of affairs is not universal and it probably does not apply to the larger bakeries where there is often some system of testing before the main bulk is used for bread production.

In test baking, especially where flours milled from individual wheats are examined, the question of water absorption is of great significance since with flours milled from wheats of widely different origin and quality erroneous impressions of their baking qualities may be obtained unless the doughs contain the correct amounts of water.

When the study of the physical properties of doughs was begun in these laboratories it was realised that the water contents at which doughs were to be examined were among the first problems to be solved. Two methods were available—either all flours could be given the same amount of water or else the amount could be varied from flour to flour so that each received its optimum amount. The first method although the simplest was open to the serious objection that the doughs so examined would in many cases be in quite different physical condition from those made from the corresponding flours in the bakehouse. It

[1] One Imperial gallon of water weighs 10 pounds and 1 sack of flour weighs 280 pounds.

therefore appeared desirable to determine by what standards the optimum water content for each dough could be fixed.

The first attack on the problem was an endeavour to determine what were the impressions the baker obtained when making his doughs and how these guided him in assessing the correct amount of water to be used. This proved anything but easy as at this stage there seemed to be a complex mixture of impressions only one of which could be separated and defined, this being the impression of stickiness. The baker disliked a sticky dough and the approach of stickiness appeared to be a limiting factor in assessing the amount of water to be used.

The first attempt at standardising absorption against some physical property of the dough was therefore by measurements of stickiness (Halton and Scott Blair, 1937), but although methods of measuring this property were developed it was not possible to make them precise enough to distinguish with certainty differences in water content that were significant in the bakehouse.

The next attempt was to determine what were the variations in viscosity and elasticity modulus of doughs made from different types of flours when these were tested at the bakehouse absorptions. For this several flours were first examined in the bakehouse on a four-hour system and the optimum water content for each was determined by the baker. Using these absorptions, doughs were made in the laboratory and after four hours' fermentation their viscosities and moduli were measured. The measurements showed that while the viscosities of the doughs differed widely, varying with the strengths of the flours, the moduli differed only to a small degree. This proved to be a most important finding as later experiments showed that the water contents of doughs made from different flours should be adjusted so that the doughs had similar values of elastic modulus; this is necessary not only for the comparison of such flours in the bakehouse, but also for the comparison of the physical properties of the doughs in the laboratory.

In previous papers (Halton and Scott Blair, 1937) the theory was advanced that a high viscosity in dough is desirable since this property largely determines dough stability, *i.e.*, its tendency not to flow out under its own weight, while on the other hand, with modulus a low value was essential for proper expansion of the dough both during fermentation and, more particularly, when the dough is first placed in the oven. The results of experiments were given which showed that changing water content affected both viscosity and modulus in the same way; *i.e.*, increased water content lowered both viscosity and modulus while decreasing the water content raised them both.

Thus, while decreasing the water content had the beneficial effect of raising the viscosity it had at the same time the undesirable effect of

raising the modulus; and for opposite reasons, increasing the water content was desirable from the point of view of modulus but undesirable from the standpoint of viscosity. Thus, optimum absorption is to some extent a compromise between high viscosity and low modulus and

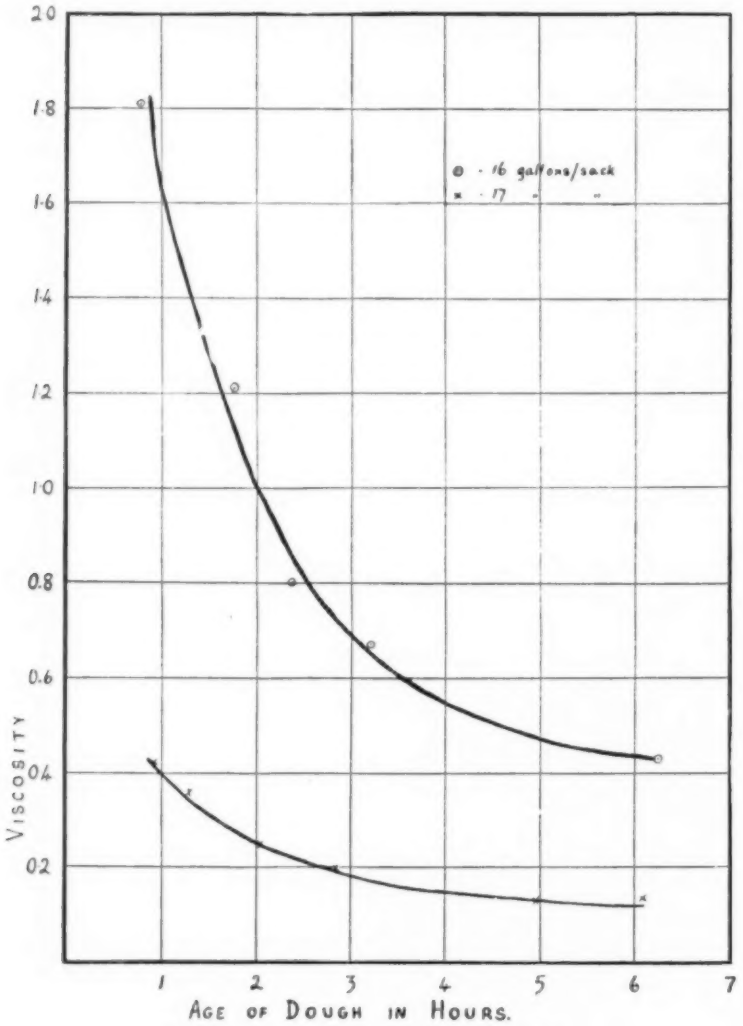


Figure 1. Change in viscosity of dough with age.

is determined by the optimum balance between the two. The balance is, however, not equally adjusted since, as has already been stated, correct absorption corresponds much more nearly to a constant modulus than it does to a constant viscosity.

The viscosity and modulus of a dough are not only affected by changing the water content of the dough, they are also affected by its age. When dough is allowed to stand, either in the presence or absence of yeast, the values of viscosity and modulus both fall with increasing

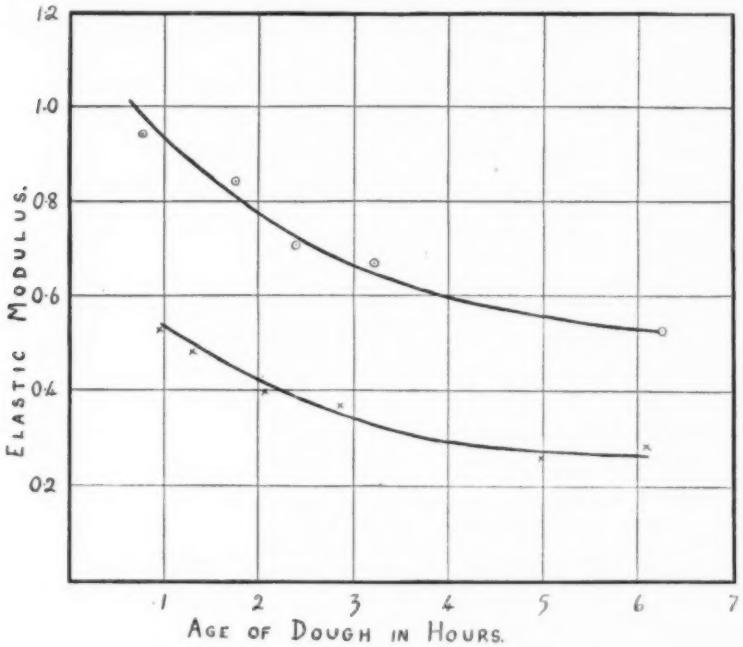


Figure 2. Change in elastic modulus of dough with age.

time.[2] The decrease is, however, not the same in both since the viscosity decreases more rapidly than the modulus, with the result that the ratio between the two is upset. This is illustrated by the data given in Table I [3] where the viscosities and moduli obtained at hourly intervals on two doughs containing 16 and 17 gallons of water per sack respectively, but both made from the same sample of No. 3 Manitoba, are tabulated. From this table it can be seen that the viscosity of the 16-gallon dough fell from 1.66 to 0.43 between the first and sixth hours (that is, by 1.23 or 74%) while the modulus fell during the same interval from 0.94 to 0.53 (or by only 43%). In the case of the 17-gallon dough the viscosity fell from 0.41 to 0.12 (or 71%) and the modulus from 0.53 to 0.26 (or 51%).

[2] This is complicated by any mechanical treatment the dough may be given during aging, but this does not upset the argument advanced in this paper. The effect of such treatment will be given in a later paper.

[3] The hourly figures for viscosity and modulus given in this table are taken from the smooth curves in Figures 1 and 2 where the measured values of $\eta$ (the viscosity) and $n$ (the modulus) are plotted against the age of the doughs.

TABLE I

VISCOSITY AND ELASTIC MODULUS DATA OBTAINED IN DOUGHS MADE FROM A SAMPLE OF NO. 3 MANITOBA

| Water content | | Age of dough (hours) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 16 gal. per sack | Viscosity ($\eta$) | 1.66 | 1.01 | .68 | .54 | .46 | .43 |
| | Modulus ($n$) | .94 | .78 | .66 | .60 | .56 | .53 |
| | $\eta/n$ | 1.8 | 1.3 | 1.0 | .9 | .8 | .8 |
| | $\eta/n^2$ | 1.9 | 1.7 | 1.5 | 1.5 | 1.5 | 1.5 |
| 17 gal. per sack | Viscosity ($\eta$) | .41 | .26 | .19 | .15 | .13 | .12 |
| | Modulus ($n$) | .53 | .42 | .34 | .30 | .27 | .26 |
| | $\eta/n$ | .8 | .6 | .6 | .5 | .5 | .5 |
| | $\eta/n^2$ | 1.5 | 1.5 | 1.6 | 1.7 | 1.7 | 1.8 |

*Note.* The above viscosity and elastic modulus measurements should be multiplied by $10^6$ and $10^4$, respectively, to express them in C.G.S. units. Therefore the ratios of $\eta : n$ should be multiplied by $10^2$ and the ratios of $\eta : n^2$ by $10^{-2}$. However, for the sake of simplicity these factors are ignored throughout this paper.

In Figure 3 the viscosity figures given in Table I are shown plotted against the corresponding modulus figures, both in the case of the 16- and the 17-gallon doughs. It will be noticed that the sets of points so obtained both fall on the same smooth curve. This points to the very important fact that the relative change in viscosity and modulus brought about by age is the same as that caused by increased water content. This is also shown up by the following data taken from Table I:

| | 16 gallons per sack | | 17 gallons per sack | |
|---|---|---|---|---|
| | 1 hour | 6 hours | 1 hour | 6 hours |
| Viscosity ($\eta$) | 1.66 | 0.43 | 0.41 | 0.12 |
| Modulus ($n$) | 0.94 | 0.53 | 0.53 | 0.26 |
| $\eta/n$ | 1.77 | 0.81 | 0.77 | 0.46 |

From the above it can be seen that for the 16-gallon dough at 6 hours the values for viscosity, modulus and the ratio of the two had fallen to values similar to those obtained at 1 hour with the extra gallon of water. If a dough containing 16½ gallons per sack had been examined the viscosity-modulus curve for this would have overlapped those of the 16- and 17-gallon doughs.

## A New Method of Test Baking

In a previous paper (Halton and Scott Blair, 1937) it was suggested that the tolerance of a flour to changing fermentation time was de-

pendent, at least in part, on the change that takes place in the viscosity and modulus, and particularly in the ratio of the two, with changing time. If this is true it would follow from the experiments just quoted that the tolerance of a flour to changing time should be similar to its

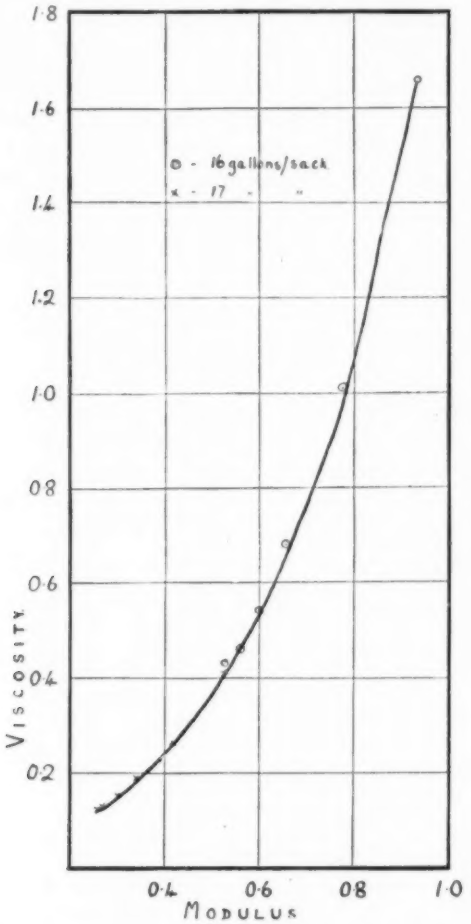


Figure 3. Relationship between viscosity and modulus measurements made on doughs from a sample of No. 3 Manitoba flour.

tolerance to varying water content. This has now been confirmed by tests made in these laboratories over a period of more than a year, and has resulted in a new system of test baking being adopted as our standard procedure.

In our older method (see Fisher and Halton, 1937) it was customary to make at intervals of three-quarters of an hour a series of doughs from the same flour, each having the same absorption. These were fer-

mented and placed in the oven at the same time. As a result a series of loaves were obtained having different fermentation times so that the improvement up to, and the deterioration after, the optimum time were shown up. With a flour of good tolerance a number of similar and good loaves were obtained while when the tolerance was poor one best loaf only was obtained, those having shorter or longer fermentation being inferior. With experience, and a prior knowledge of the type of flour being tested, it became possible to reduce the number of loaves baked and often only three were necessary.

To test the theory that tolerance to time and water were similar two extra loaves were included in the series each having the same fermentation as the middle loaf in the changing time set but one having an extra gallon and the other a gallon less per sack than the standard. This was called "baking the flour on the cross" since, for convenience of comparison, the loaves were arranged for examination as in the following example:

| | 16 gallons<br>5½ hours | |
|---|---|---|
| 15 gallons<br>4¾ hours | 16 gallons<br>4¾ hours | 17 gallons<br>4¾ hours |
| | 16 gallons<br>4 hours | |

This test has now been made on a large number of flours and it has been found that whenever the tolerance to time is good the tolerance to water is also good and *vice versa*. With Manitoba flours, for example, not only are three good and similar loaves obtained on the varying time system but three good and similar loaves result from changing the water content. With English, or other flours having poor tolerance, the centre loaf is usually the best and similar degrees of deterioration are shown by increase and decrease of water as by increase and decrease of time. The details of times and water contents are arranged to obtain the best loaf as the centre one, but this is not invariably successful. Sometimes the loaf with the shorter or with the longer fermentation time is the best of the changing time set but in such cases the corresponding loaf with the lowest or with the highest water content is also the best of the changing water series.

In general the loaf with the lowest water content corresponds to the loaf with the shortest fermentation time, while that with the highest absorption matches that with the longest fermentation. Although these corresponding loaves are generally very similar an exact match is not to be expected. This is because the quality of the bread is dependent on other factors besides the viscosity and modulus of the dough. It is, for example, influenced by the rate of gas production, particularly during the final proof. The gas-production curve of a flour approaches linearity over only a comparatively short interval of time with the result that three doughs differing in age by ¾ of an hour and 1½ hours respectively may also differ in their rate of gas evolution. Gas production is also influenced by water content of the dough although only to a very small extent, with the result that gassing is a much less variable factor in the "changing water" than in the "changing time" series. The effect of this gas-production factor varies from flour to flour, but in general exact correspondence between any two loaves can only be expected when the rate of gas production as well as the physical properties is the same in both cases.

A difference in rate of gassing may be reflected in a variation in loaf volume which in turn may affect crumb structure. Corresponding loaves may also differ in the character of the crust, particularly in colour. This is because in a series of doughs fermented for increasing lengths of time the amount of sugar used up in gas production increases with time and, even allowing for the continued production by diastatic action, the amount of residual sugar falls. As a result the crust colour, which is largely influenced by the sugar content of the dough at baking, becomes lighter as fermentation increases. With doughs of varying water content but the same length of fermentation the sugar contents at baking are similar and therefore the crust colours are similar.

It is therefore not to be expected that the corresponding loaves in the changing time and changing water series should necessarily be identical. However, it must be pointed out that these differences are only small and in any case do not affect the judgment of baking quality. The object of either system of testing is to determine the optimum quality of bread a flour is capable of producing and its fermentation tolerance. In either system a series of loaves is baked with one variable (either time or water) being altered from loaf to loaf and in this way the conditions for optimum quality are found. Thus with either test the best quality bread is produced somewhere in the series, and since the tolerance to water is similar to the tolerance to time, the same general picture of baking quality results in both cases.

Another possible difference between loaves in the two series can be caused by variation in amount of bakehouse manipulation. While

doughs having varying water contents but the same fermentation time receive the same number of mouldings and knocks back, those with different fermentation times may receive different amounts of treatment.

A third factor which has to be taken into account is the shortness factor, but while the significance of this, as also that of bakehouse manipulation, must be left for future consideration, it can be pointed out that neither materially upsets comparison by the two methods of testing.

In general when 2% of yeast is used loaves baked at ¾-hour intervals of time give a similar picture of flour quality and tolerance to those baked with 1-gallon differences in water content so that, roughly speaking, 1 gallon is approximately equivalent to ¾ of an hour. From measurements of viscosity and modulus made with varying time and varying water content it would appear that 1 gallon was equivalent to very much more than ¾ of an hour. The reason for this disagreement is not at the moment understood but it must be due to some of the other factors that enter into the determination of bread quality, *e.g.*, oven factors.

As a result of the comparison of the changing time and changing water methods over a considerable period of time, the latter method has been adopted in place of the older method, because of certain advantages the new method has over the old.

With the changing time method the assessment of the correct absorption is in the hands of the baker and this assessment is not easy with certain types of flour. As a result, incorrect deductions are likely to be made from the baking test, errors which are avoided with the changing water method since with the range of water contents used the correct absorption is invariably included among them.

Another advantage of the new method is the smaller variations in gas production that occur compared with the varying time method. This eliminates an error of judgment that is possible when testing poor gassing flours or those that need a long development time on the variable fermentation method. This error is likely to occur because of the falling off in quality of the loaf having the longest fermentation being due, in part or wholly, to a falling off in gassing rather than to a falling off in physical properties. Even when sugar is added gassing is not always prolonged sufficiently to cover the whole range of fermentation times used in the test.

The ideal is that all doughs should gas not merely adequately but at the same rate and this is very nearly so when the water content only is varied.

### Optimum Absorption and Optimum Fermentation

Since fermentation time and water content are to a large extent interchangeable in their effects, the terms optimum or correct absorption and optimum or correct fermentation are only relative and not absolute for any particular flour. Thus, if it is desired to produce the best quality bread from a flour at say six hours, the water content of the dough should be less than if the fermentation time were only 3 hours. For similar bread to be made the physical properties of the doughs should be the same at the ends of the two periods, and since in the case of the 6-hour dough softening proceeds for a longer time, this dough requires less water at making than does the 3-hour dough. For the same reason, doughs made from the same flour but having different water contents require different fermentation periods if each is to bake into the same quality bread.

It is thus clear that correct absorption and correct fermentation times are interrelated, and a baker when making his doughs should assess their absorptions not only on the types of flour he is using but also on the length of fermentation he intends to give them.

### The Softening of Dough with Age

This has been generally considered as being due to enzyme action (see Jørgensen, 1936), but while probably partly true in the case of doughs containing flour from sprouted wheats or wheats damaged by frost before maturity or attacked by the wheat bug, the experiments quoted earlier in this paper suggest a different mechanism equally as possible in the case of flours milled from sound wheat.

Many protein structures when in a hydrated condition exhibit the phenomenon known as "syneresis." This can be observed to occur with a piece of moist gluten that is covered by an inverted glass beaker to prevent drying, the surface of the gluten becoming gradually covered with drops of water.

Since on aging the viscosity and modulus decrease in the same manner as they do when extra water is added to the dough, it is feasible to suggest that the softening of dough with age is due to syneresis and that the water given out by the protein acts in the same way as added water in "diluting" the dough.

Much interesting information bearing on this softening of dough with age has been obtained and it is proposed to discuss this in a further communication.

### Viscosity-Modulus Relationship and Water Content

The experiments quoted earlier in this paper have not only led to a new system of test baking but have also thrown new light on the

importance of the viscosity-modulus relationship as a determining factor in dough quality.

In an earlier report (Halton and Scott Blair, 1937) experiments were described from the results of which a theory was developed concerning the functions of viscosity and elastic modulus in dough behaviour. It was suggested at the same time that the relationship of viscosity to modulus was the best criterion of the strength of a flour since those milled from such wheats as Manitoba had the highest, while English and other soft wheat flours had the lowest value for the ratio of $\eta/n$.

It was found that due to the differential effect on viscosity and modulus of age and water content, the simple ratio of $\eta/n$ was not a constant and comparisons of this ratio for different flours were therefore always made on doughs having the same value for $n$.

It has since been found that the viscosity-modulus curve, as in Figure 3, is unique for a particular flour; that is, each flour has its own curve which differs in regard to its placings relative to the $\eta$ and $n$ ordinates from the curve of any other flour. It appeared possible that some other relationship such as $\eta/n^x$, where $x$ is some unknown quantity, might be a constant irrespective of dough age or water content.

$$\text{If} \quad \eta/n^x = k \text{ (a constant)}$$
$$\text{then } \log \eta = x \log n + k_1$$

and the curve obtained by plotting $\log \eta$ against $\log n$ should be linear with a slope of $x$.

In Figure 4 the logarithms of the corresponding values of viscosity and modulus given in Table I are plotted against one another. The points so obtained while not lying on a straight line, closely fit one, the slope of which is 2. This shows that the ratio $\eta/n^2$ has a value which is almost independent of dough age or water content. In Table I the values of $\eta/n$ and $\eta/n^2$ are given for each hour at the two water contents and it can be seen that whereas the value of $\eta/n$ varied from 1.8 and 0.5, the value of $\eta/n^2$ only varied between 1.9 and 1.5.

In the earlier work comparisons of the values of the simple ratio $\eta/n$ were made at a value of $n = 1$. For each flour a dough was made having a modulus rather greater than 1 and measurements of $\eta$ and $n$ were made at intervals during the aging of the dough. Curves were then drawn relating $\eta$ and $n$ to the age of the dough and from these the value of $\eta$ corresponding to $n = 1$ was read off. The absorptions required to give doughs having this value for modulus were found to be related to those required for the production of optimum quality bread in the bakehouse and experience showed that the values of $\eta/n$ so obtained agreed satisfactorily with the relative strengths of the flours tested.

The slope of the log $\eta$/log $n$ curve is not exactly 2 for all flours but in the majority of cases varies between 1.8 and 2. With all flours the log $\eta$/log $n$ curve is either a straight line or as in Figure 4 very nearly so, so that in every case $\eta/n^x$ can be taken as a constant even though the

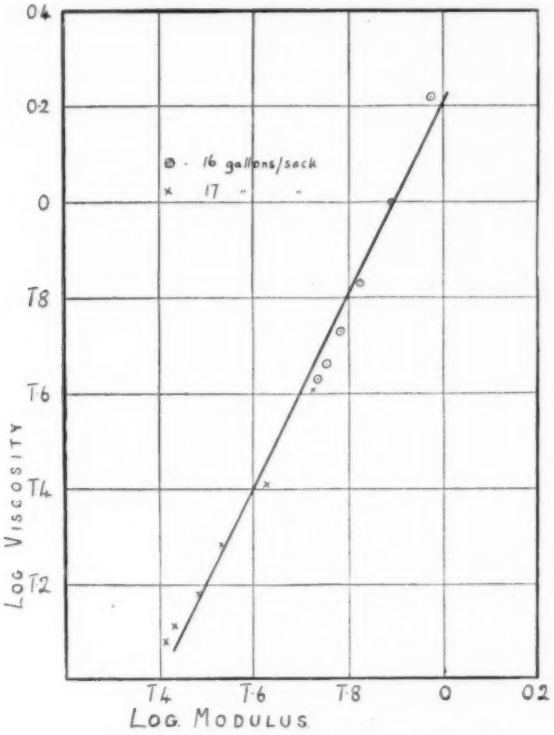


Figure 4. Relationship between the logarithms of viscosity and modulus measurements made on doughs from a sample of No. 3 Manitoba flour.

value of $x$ may vary slightly from flour to flour. This does not upset the convenience of using $\eta/n^x$ as a measure of flour strength since the value of $\eta/n^x$ is the same as that of $\eta/n$ when $n = 1$ and, as has already been stated, the justification for using the latter has been proved by experience.

At the moment our knowledge of the mechanism of the viscous and elastic properties of dough is insufficient to give any reason why the value of $x$ in $\eta/n^x$ should or should not be the same for all flours, but the fact that for any particular flour $\eta/n^x$ is a constant shows that $\eta$ and $n$ are not just two independent variables but that even though they are affected to different degrees by dough age or changing water content, there is some definite relationship between them irrespective of the condition of the dough.

## Summary

The effect of aging of dough on the ratio of viscosity to elastic modulus has been found to be the same as that produced by increasing the water content of the dough. An inference from this, which experiment has proved correct, is that the tolerance of a dough to changing water content is the same as its tolerance to changing fermentation time. Based on this a new system of test baking has been devised.

Optimum water absorption and optimum fermentation time are not absolute for any particular flour but are functions of each other.

The value of $\eta/n$ varies with the age and water content of the dough but the ratio $\eta/n^x$, where $x$ has a value of 1.8 to 2.0, is a constant for any given flour and varies only with the quality of the flour used; it is independent of dough age or water content. The value of this ratio is therefore the best relationship of $\eta$ to $n$ to use as a measure of flour strength.



## Acknowledgment

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# SOME PHYSICAL AND CHEMICAL PROPERTIES OF EGYPTIAN BREAD

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Bakers Weekly, 45 West 45th Street, New York, New York

(Read at the Annual Meeting, May, 1937)

In 1935–36 the Metropolitan Museum of Art Egyptian Expedition, headed by Ambrose Lansing and William C. Hayes, carried on excavations in the Asasif Valley on the site of the Ramesside temples and on the upper slopes of the Sheikh Abd el Kurneh Hill, in the interests of Egyptology. After much disappointing work they finally uncovered some valuable material that is discussed by Lansing and Hayes (1937) in a Bulletin of the Metropolitan Museum of Art.

In carrying on clearing work on the west side of Thebes the expedition came to some scant remains of the mortuary temples of Rameses IV, V and VI, and other material that represents the life of the 12th and 25th dynasties. In the debris of the Ramesside temples was found a head representing Amen-Hotpe I, of the 18th dynasty, whose statue had probably once stood in the Avenue of Mentu-Hotpe.

Amen-Hotpe, together with Sen-Mut, Hat-Shepsut, Ra-Mose and Hat-Nufer, plays a prominent part in the expedition's findings which relate to the bread discussed in this paper, as well as other substances with which the authors are not concerned at this time.

It appears that Hat-Shepsut was the reigning queen of the period who had as one of her favorites the architect and builder of the day, Sen-Mut, who was the son of Ra-Mose and Hat-Nufer. In the picture also appears Amen-Hotpe who, so far as can be learned, was related to the king who reigned prior to Hat-Shepsut. She acquired the ruling power, which in all probability belonged to Amen-Hotpe, and since he was a child at the beginning of her reign she apparently had no difficulty in exercising her will upon the people of Egypt.

It also appears that life was carried on very much the same as it is today. Models of granaries and a model of a brewery and a bakery housed in the same building, now in the possession of the Metropolitan Museum of Art, indicate that grain was harvested, threshed, ground into meal and made into various products very much the same as it is in our day. The model of the granary obtained by the expe-

[1] The authors are indebted to Andrew Dingwall for the assistance given and work rendered in the spectrographic work, and to Charles Shillaber for his work in relation to the photomicrographs.

dition shows the Egyptian workers carrying the grain to be measured and stored for future use.

The model of the brewery and bakery shows organized effort which is also quite similar to present-day operations in the arts of brewing and baking. In the brewery the grain was ground and made into a paste or dough and then molded into loaves in very much the same manner as dough is molded in the bakery. This dough was then baked, apparently to obtain gelatinization of the starchy material and coagulation of the protein substance. These loaves, after drying, were ground into a meal which was then mixed with sprouted grain, probably barley, and the mashing process was carried on to obtain conversion of the gelatinized starch into sugar. The mash was then leached by depositing it onto leaves of the sycamore tree that had been spread over gratings. In this way they seemed to have obtained filtration and, at the same time, tannin from the leaves. This wort was then deposited into wooden casks resembling our large fermentation vats, but of much smaller size, where apparently the fluid was fermented. From here it was placed in stone jars and sealed with lids.

The model of the bakery which lies adjacent to the brewery closely resembles the latter; and in the bakery the grain was crushed in the same way and made up into dough. In the operations the dough was then divided into loaves, molded, fermented, and baked in rectangular ovens which in appearance were on the order of our so-called peel-type ovens. Cakes were baked in cylindrical ovens resembling the rotary ovens of today but without a rotating hearth.

Charles Martyn (1927) presents in his record a picture of the foods and culinary utensils used by the ancient Egyptians. The reported history of Egypt, which according to Herodotus was known as Thebes, commences with the reign of Menes, or Menas, who was supposed to have been its first king. He ascended the throne about 2320 B.C. The growth of civilization in Egypt was much more rapid than in any contemporary nation. The early life was devoted to agriculture and the rearing of cattle. Goats were kept for milk. Beef and goose were more generally eaten than any other kind of meat. Lower Egypt used dried and salted birds, such as geese and ducks, as well as many varieties of fish. The Egyptians had frequent banquets and all meat used was freshly slaughtered. They made macaroni and used bronze cauldrons, some alloyed with tin and iron.

The Egyptians were fond of a great variety of cakes and dainty confections. The more elaborate forms of pastry were mixed with fruits and seeds and shaped to represent animals, birds and human beings. The plainer rolls were generally mixed by hand and sprinkled with seeds before baking. At other times they were prepared from a

thinner mixture, well kneaded in large wooden bowls (the feet were often used for this purpose) and then carried in vases to the chief pastry cook who formed it into a sort of macaroni upon a metal pan over the fire, stirring the mixture with a wooden spatula, while an assistant stood ready with two pointed sticks to remove it when sufficiently cooked.

Wine and water were placed in porous jars and fanned until cool. The water was purified by the use of a paste of almonds.

The vegetable kingdom of the Egyptians may be roughly divided into four classes: Trees and shrubs; succulent plants; grains; and various grasses. The most important food-producing trees were the doom and date palms, the sycamore and mokhayp. The wood of the doom palm was used to build boats and the fruit was eaten both ripe and unripe. In the latter state it had about the texture of cartilage and in the former it was similar to the edible portion of the coconut.

Dates were used for making brandy, wine and vinegar. An exhilarating drink was also made from the sap of this tree. The fruit of the sycamore ripened in June and was much esteemed by the Egyptians. The fruit of the mokhayp was used extensively as a medicine and according to Pliny was made into a fermented liquor. Other fruit trees and shrubs include the fig, pomegranate, olive, peach, pear, plum, apple, grapes, etc. The olives were large and fleshy but contained little oil. The juice of the grape was sometimes drunk in the fresh condition but fermentation was usually awaited and the wine stored away in sealed vessels.

The succulent plants included the byblus, papyrus, lotus and nelumbo. Papyrus is no longer found in the country. It grew mainly in marshy districts. The pith of the upper and middle portions was used for paper but the lower portion and the root were considered an edible delicacy.

Wheat and barley were grown in all provinces of the valley and, to a lesser degree, rice, millet, peas, beans, lentils, etc.

The bread and cakes used in the homes of the wealthy were made from wheat flour. Those one degree lower in the social scale used barley meal and the poorer classes ate bread of durrha (holcus sorghum) flour.

Grüss (1928, 1929) has reported the composition and physical characteristics of the grain used by the Egyptians in the art of brewing. Nothing of any importance has been found in the literature in regard to the composition of the breads used by the Egyptians of the 18th dynasty other than the study of Whymper (1913) in "The Influence of Age on the Vitality and Chemical Composition of the Wheat Berry." He comes to the conclusion that the baked material was put into the

tombs of the dead for the purpose of furnishing them with refreshment in their journey to the world "beyond the sunset." He also concludes that the baked products were subject to decay due to the fermentable property of the starch and sugar with the result that for the most part only the cellular tissue remained.

This seems to be a general belief and probably for this reason the writers were unable to find in the literature any record of a study having been previously made on these materials. In the case of wheat grains, however, Whymper finds that age has not wrought a very great change. The lowering of the ash content and an increase of acidity are the two marked changes as compared with present wheat but he was unsuccessful in an attempt to germinate the wheat.

The thought may be injected here that to the authors' minds it seems logical that the ancient Egyptians might have deposited these foodstuffs and architectural models into the tombs as a means of preserving for posterity their knowledge and accomplishments.

In cooperation with Lansing samples were obtained of seven products taken from the tombs of Amen-Hotpe, Ra-Mose and Hat-Nufer, which in every respect resemble grain foods of some kind with the possible exception of the product obtained from the tomb of Ra-Mose and Hat-Nufer which appears to be a combination of fruit, largely raisins, which has been charred.

Figures 1 to 3 illustrate the various products obtained in the tombs mentioned.

In Figure 1 Box *A* is apparently a dark breadstuff somewhat resembling in physical characteristics our rye bread. It appears to have been baked in a cylindrical, cone-shaped vessel made of earth. Box *B* is a light, porous product, its physical characteristics being on the order of our present-day honey cake or honey bread. The Egyptians made this product into the images of animals and men. Box *C* is a hollow product having a sort of finger shape similar to our eclairs but made up of grain and possibly other materials. In Figure 2 Box *D* appears to be a residue of mash made up of grain and either raisins or grapes, probably the latter because of the coloring and the character of its composition. This is probably a press residue that remained in the manufacture of wine. It is well known that the Egyptians made wine. Box *E* is a large, somewhat heavy piece resembling bread, with a brownish red color indicating the use of barley or some similar cereal. Box *F* is apparently a grain residue remaining in the process of manufacturing beer. It is largely hulls of grain in which is embedded a leaf of sycamore, as can be seen.

Figure 3 (Box *H*) represents a product on the order of our plum pudding, according to Lansing. It is rather heavy, showing very


Figure 1


Figure 2


Figure 3

little porosity, is quite charred and has the appearance of being made up of a deposit of fruit with a small quantity of grain to hold it together.

Table I records the analysis of the various products mentioned. It will be seen that the moisture is low in comparison with present-day trends but in keeping with the cakes and so-called hard sweets made by the biscuit and cracker industry of today. Of course, the moisture content obtained by the authors may not be the true moisture content due to the long period of storage.

TABLE I

CHEMICAL ANALYSIS OF EGYPTIAN BREAD

| | Amen-Hotpe Box *A* | Amen-Hotpe Box *B* | Amen-Hotpe Box *C* | Amen-Hotpe Box *D* | Amen-Hotpe Box *E* | Amen-Hotpe Box *F* | Ra-Mose and Hat-Nufer Box *H* |
|---|---|---|---|---|---|---|---|
| | % | % | % | % | % | % | % |
| Moisture | 7.88 | 7.90 | 8.22 | 9.26 | 8.24 | 6.88 | 9.62 |
| Ash | 7.78 | 4.96 | 4.20 | 3.28 | 3.34 | 13.30 | 0.36 |
| Nitrogen | 2.40 | 2.38 | 2.085 | 2.705 | 2.322 | 3.035 | 0.825 |
| Protein (N × 5.7) | 14.13 | 13.57 | 11.88 | 15.42 | 13.25 | 17.31 | 4.71 |
| Fat | 2.595 | 2.425 | 2.595 | 0.62 | 1.70 | 0.48 | 3.26 |
| Crude fiber | 2.26 | 0.98 | 2.66 | 4.02 | 1.64 | 16.14 | 0.60 |
| Salt (Na CL) | 1.75 | trace | 0.627 | 0.0163 | 0.288 | 0.0407 | trace |
| Ash not soluble in $HNO_3$ | 2.32 | 0.18 | 0.8 | 1.60 | 0.32 | 7.98 | 0.02 |
| Dextrin and sugar | 68.0 | 62.0 | 52.0 | 40.0 | 65.0 | 32.0 | 30.0 |
| Reducing sugars | — | — | 0.450 | 7.11 | 0.501 | — | 21.94 |
| Sucrose | — | — | — | 3.42 | — | — | 0.827 |
| pH value | 4.19 | 5.10 | 4.47 | 3.98 | 4.31 | 4.94 | 3.60 |

The ash is high, especially in some of the products, probably due to their having been baked in earthen vessels and also having come in contact with earth. This can be said of sample *A* in Figure 1. The upper crust had a heavy deposit of what appeared to be ash.

The nitrogen, when multiplied by the protein factor, gives a protein content equal to that of the protein of cereals today. The fat, too, is in keeping with some of our yeast-leavened goods. The fibre indicates that whole grain was employed in the manufacture of the substances.

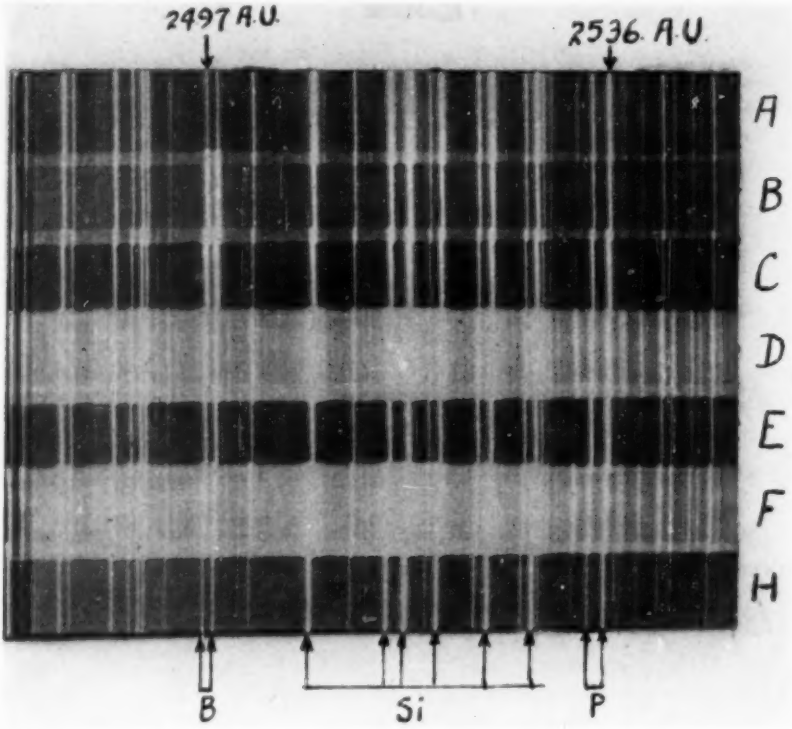


Figure 4

The chlorine, calculated as sodium chloride, varies considerably from just a trace to 1.75%. The ash in sample *A* (Figure 1) and in sample *F* (Figure 2) is very high.

The authors carried on some work to determine the quantity of sugar present and, in an effort to conserve the sample, came to the conclusion that it would be best to work with a polariscope. It was soon discovered that only two samples gave a very slight starch reaction, and in the polarization work as well as in the gravimetric work

large quantities of dextrin were found in each sample. Consequently it was necessary also to run a sugar determination gravimetrically. Since the polarization was much higher than the quantity of sugar obtained by direct analysis, and the qualitative test indicated dextrin,

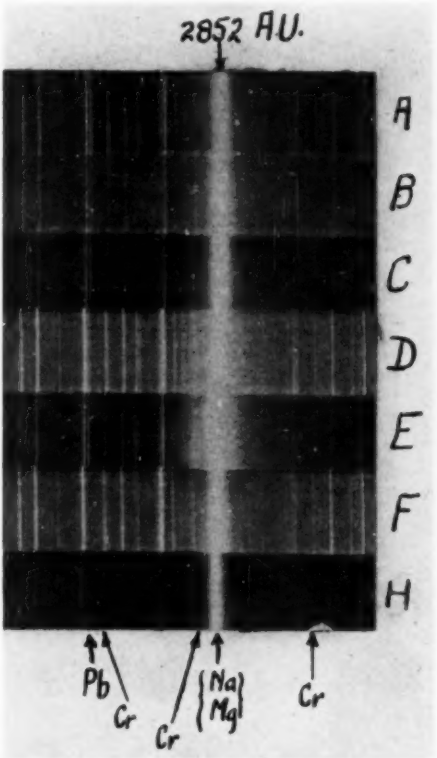


Figure 5

the figure obtained was designated as dextrin and sugars. This indicates that the starch had been converted into dextrin, which is quite possible as the samples had been stored since 1494 B.C.—almost 3,500 years.

All of this material is of the 18th dynasty, during the reign of Queen Hat-Shepsut, the date having been fixed from inscribed objects found in the tombs of Ra-Mose and Hat-Nufer. The conversion of the starch into sugar by heat over a long period of time is easy to assume. The temperature within the tomb where this material was discovered is somewhere around 110° F., and in resting over a period of almost 3,500 years it is quite possible that all of the starch material would become dextrinized.

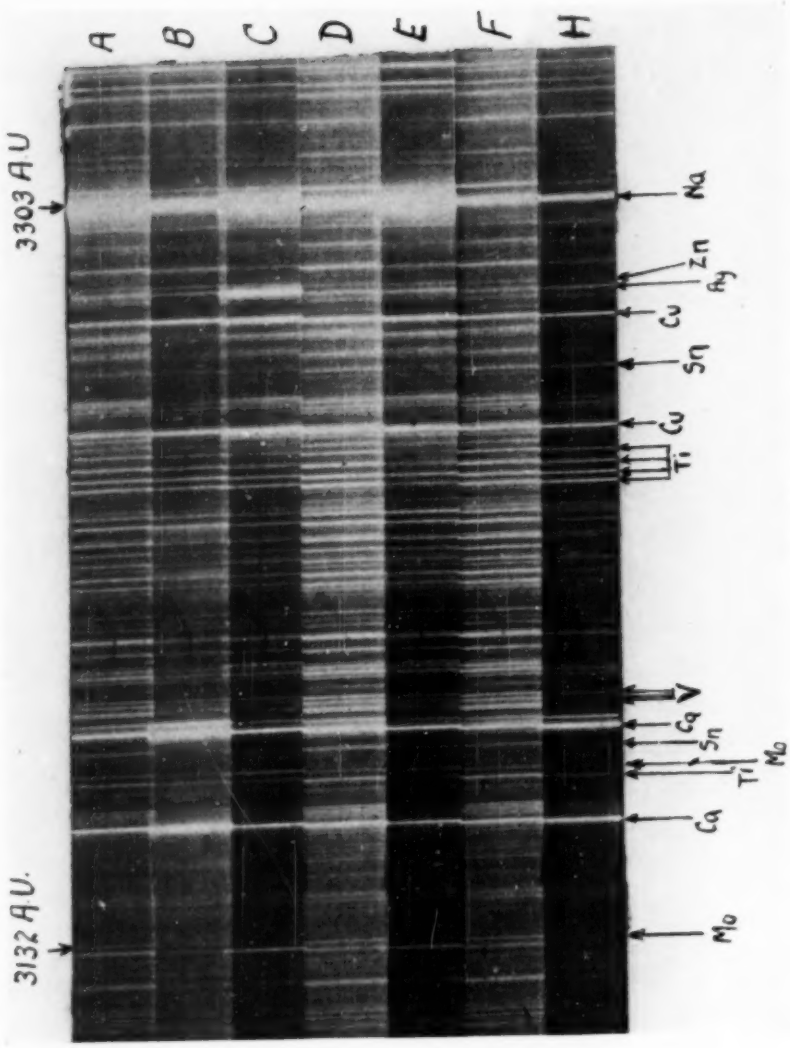


Figure 6

The dextrinization of starch has been reported by Bouillon-Lagrange (1904) and he published his method of dextrinization of starch in 1911.

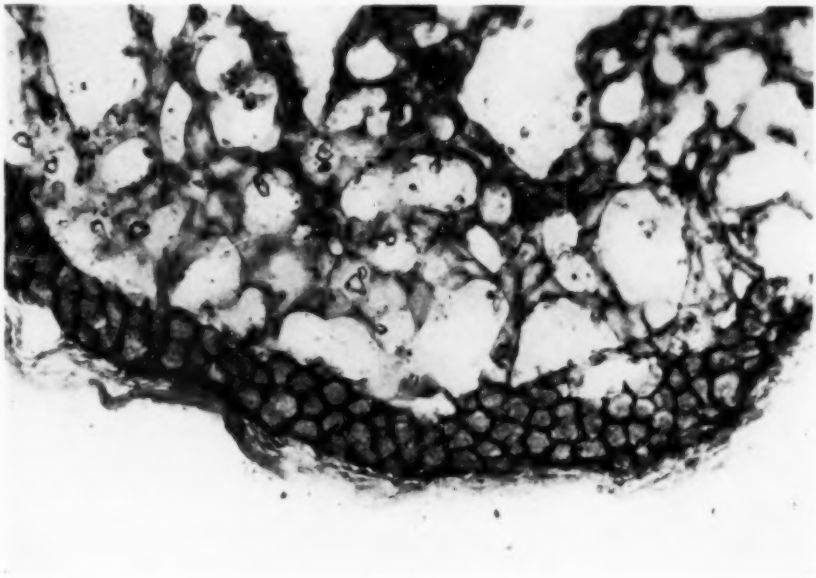

Figure 7

The method used in making these various determinations follows:

*Moisture:* Determined according to the A. A. C. C. method for baked cereal products.

*Ash:* Determined according to official A. A. C. C. method for baked cereal products.

*Nitrogen:* Determined according to the A. A. C. C. method for determining nitrogen in baked cereal products.

*Protein:* Percentage of protein obtained by the use of the factor 5.7.

*Fat:* Determination was made following the A. A. C. C. ammoniacal alcohol method for baked cereal products.

*Crude fibre:* Determined in accordance with the official A. A. C. C. method for cereal products.

*Chlorides:* Determined by following the A. A. C. C. method for determining sodium chloride in the ash of cereal foods.

*Dextrins; Sugars:* So designated due to the fact that a qualitative dextrin determination on the products gave a positive result, and in the attempt to determine sugars by polarization according to the A. A. C. C. method for polarizing sugars the results obtained were too high to be indicative of the sugars alone.

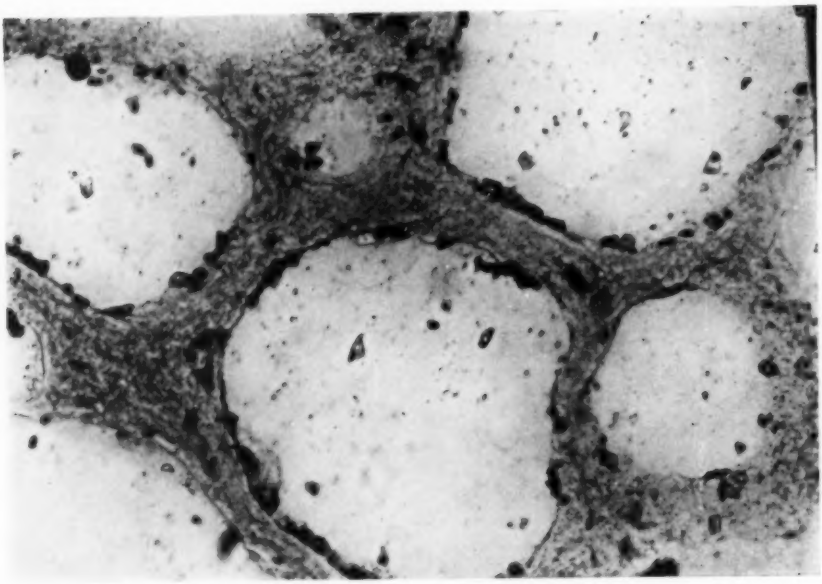

Figure 8

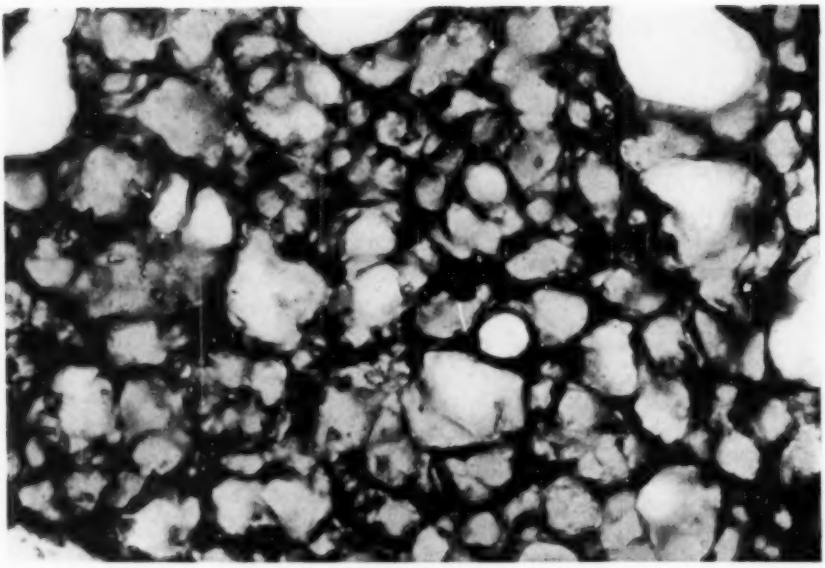

Figure 9

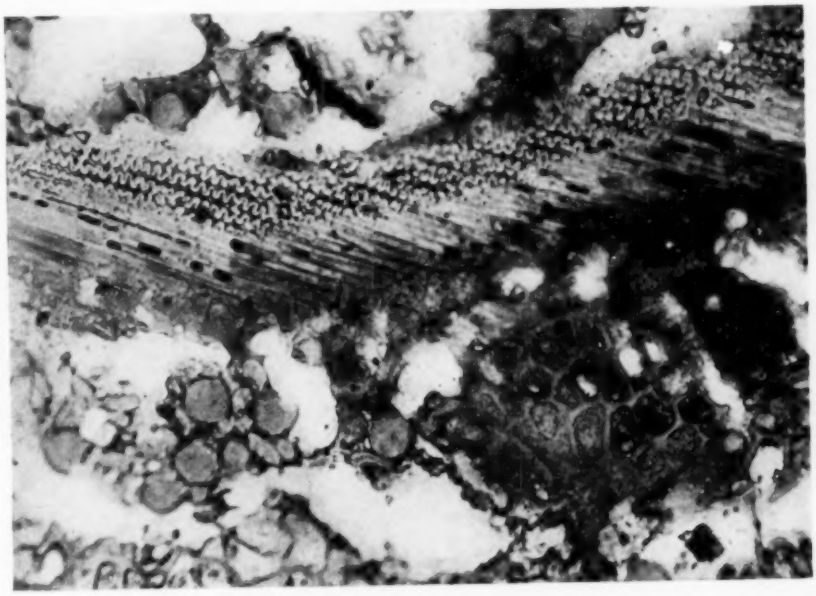

Figure 10

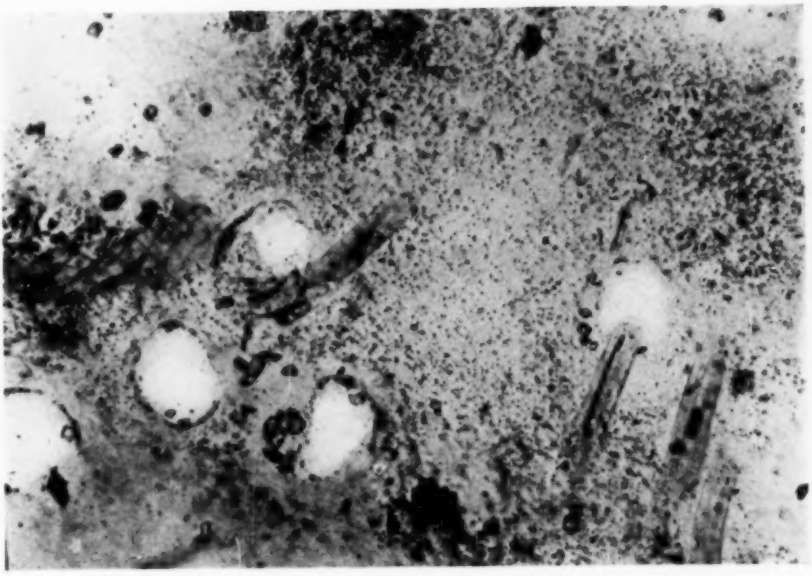

Figure 11

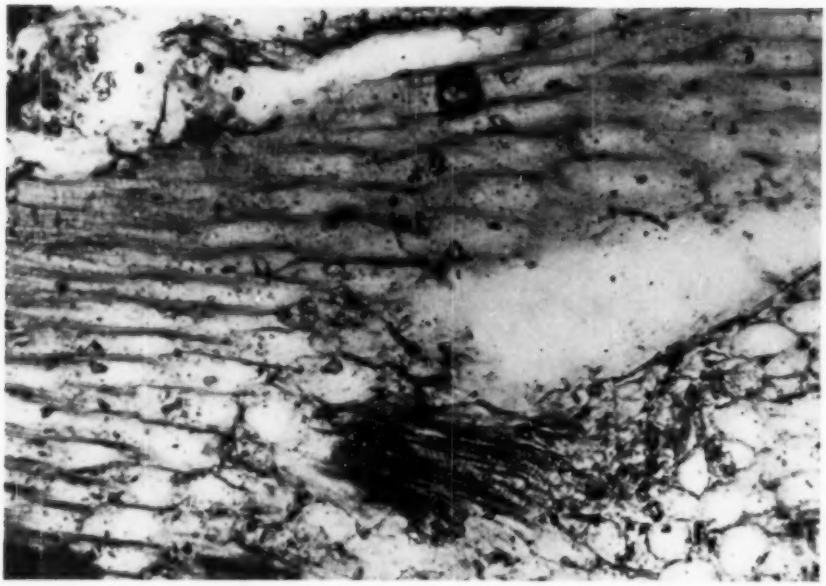

Figure 12

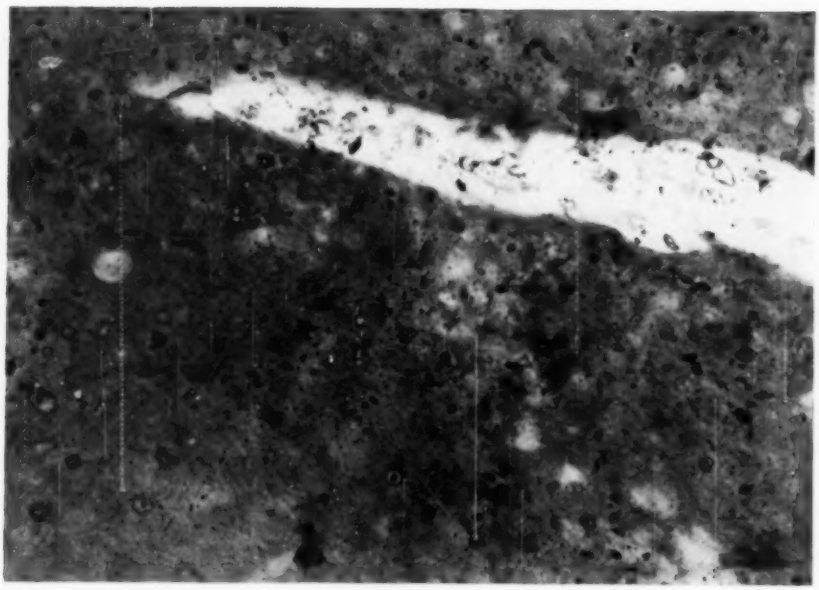

Figure 13

*Reducing Sugars and Sucrose:* Determined according to the Munson-Walker method for determining these on cereal foods.

In addition to making a chemical analysis, a spectrograph of the minerals contained in these breads and cakes was also obtained. Silver, boron, copper, tin and lead were found. Samples *A*, *D* and *F* contained rather heavy deposits of lead. Of course, lead is also present in the others.

Figures 4 to 6 represent a spectrum of the ash of these materials. In addition to the spectrograph, photomicrographs were made of portions of the structure of the products, as shown in Figures 7 to 13. Difference in the structure of the photomicrographs will be noted. Figure 7 is particularly interesting in that it shows, in the lower portion, a micrograph of the crust structure and adjoining this is the crumb structure. Considerable fibre was noticeable in these various samples, excepting sample *H* which under the microscope resembled a resinous material (see Figure 13). There was a continuous field in observing this material under the microscope.

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# PHOTOELECTRIC SPECTROPHOTOMETRY APPLIED TO THE QUANTITATIVE ANALYSES OF CAROTENOID AND CHLOROPHYLL PIGMENTS IN TERNARY AND QUATERNARY SYSTEMS [1]

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(Read at the Annual Meeting, May 1937)

Recently, spectral analyses have been applied extensively to binary mixtures by Kuhn and Smakula (1931), Zscheile (1934), Miller (1934), Dingwall and Thomson (1934), and Van der Hulst (1935). This paper concerns the quantitative analyses of the carotenoid and chlorophyll pigments in ternary and quaternary systems by photoelectric spectrophotometry. For convenience, the paper is divided into (*a*) ternary mixtures of known composition, (*b*) a simple test case of unknown ternary mixtures, (*c*) known quaternary mixtures, and (*d*) unknown quaternary mixtures and the standardizing of spectral analytical method. The apparatus and method of measuring absorption coefficients have been described in detail by Miller (1937). Additional details regarding the construction, calibration, and use of a photoelectric spectrophotometer in chemistry have been published recently by Hogness, Zscheile, and Sidwell (1937).

## Ternary Systems

Following the convention suggested by Miller,[2] the effective absorption at $\lambda'$ for a ternary mixture is expressed by equation 1.

$$\alpha_T^{\lambda'} = \alpha_1^{\lambda'}\left(\frac{C_1}{C_T}\right) + \alpha_2^{\lambda'}\left(\frac{C_2}{C_T}\right) + \alpha_3^{\lambda'}\left(\frac{C_3}{C_T}\right). \qquad (1)$$

The absorption spectra reported by Miller (1935a) were employed as standards and a guide in selecting suitable wave lengths of light to be employed in analyses. As a test case of a very simple ternary system, mixtures of known composition were employed. For this, lycopene, beta carotene, and leaf xanthophyll [3] were selected because the posi-

[1] Presented at the twenty-third annual meeting of the American Association of Cereal Chemists at Minneapolis, May 27–28, 1937.

[2] The writer wishes to acknowledge and express thanks to A. E. Sidwell, Research Associate, Department of Chemistry, University of Chicago, for aid in making some of the calculations and tissue extractions employed in this study on ternary systems.

[3] Strain (1936) has shown that leaf xanthophyll consists of several isomers. Since the leaf xanthophyll employed in these mixtures was identical with that used by Miller (1935a), the use of this standard is permissible. In the subsequent paragraphs the method of standardizing the xanthophyll analyses is described. Since the author has stated the absorption coefficients at the various wave lengths that were employed as standards, it is possible to make the necessary correction for absolute analyses should the reference xanthophyll curve be too high or too low.

tions of their absorption bands occur at different wave lengths. From known stock solutions, aliquot portions were measured volumetrically and a series of mixtures was made so that in a particular series each component formed 12.5, 25.0, 33.3, 50.0, and 75.0% of the total solute. All measurements were recorded the same day these ternary mixtures were made. The analytical results of this experiment are summarized in Table I.

TABLE I

SPECTRAL ANALYSES OF TERNARY MIXTURES CONSISTING OF BETA CAROTENE, LEAF XANTHOPHYLL AND LYCOPENE

| | Known composition in per cent | | | | |
|---|---|---|---|---|---|
| | 12.5 | 25.0 | 33.3 | 50.0 | 75.0 |
| Composition found by spectral analyses at wave lengths | Composition in terms of lycopene | | | | |
| 5150 A.U. | 12.0 | 24.7 | 33.0 | 49.6 | 75.2 |
| 5200 A.U. | 11.9 | 25.4 | 33.9 | 49.8 | 75.7 |
| Average | 11.95 | 25.05 | 33.45 | 49.7 | 75.45 |
| | Composition in terms of leaf xanthophyll | | | | |
| 4600 A.U. | 12.7 | 23.6 | 33.5 | 50.3 | 75.9 |
| 4750 A.U. | 13.2 | 25.2 | 34.1 | 51.1 | 76.0 |
| Average | 12.95 | 24.4 | 33.8 | 50.7 | 75.95 |
| | Composition in terms of beta carotene | | | | |
| 4575 A.U. | 13.6 | 24.2 | 34.0 | 50.6 | 74.3 |
| 5000 A.U. | 12.0 | 24.4 | 32.9 | 49.3 | 74.5 |
| Average | 12.8 | 24.3 | 33.45 | 49.95 | 74.4 |

As an additional check that the correct analyses were obtained (Table I), λs 5200 and 4450 A.U. were found satisfactory for measuring total lycopene and the total concentration of the other two components —treated as a binary system.

## Analysis of Unknown Ternary Mixtures

An extract of ripe tomatoes was employed to further test the application of photoelectric spectrophotometry on unknown ternary mixtures. The tomato tissue was extracted by the method as outlined by Miller (1935). These analyses are reported in Table II.

TABLE II

SPECTRAL ANALYSES OF LYCOPENE, LEAF XANTHOPHYLL, AND BETA CAROTENE IN RIPE TOMATOES

(Analyses expressed in per cent on green weight basis)

| Pigment | Lycopene | | Beta carotene | Leaf xanthophyll | |
|---|---|---|---|---|---|
| Wave length employed A.U. | 5200 | 5150 | 4950 | 4750 | 4600 |
| Sample | | | | | |
| 1 | 0.00221 | 0.00223 | 0.000763 | 0.000380 | 0.000390 |
| 2 | 0.00227 | 0.00226 | 0.000752 | 0.000375 | 0.000380 |

The averages of the values reported in Table II were substituted in equation 1 for a wave length at which $\alpha_1$, $\alpha_2$, and $\alpha_3$ are of different magnitude. When this was done for 5000 A.U., the calculated value for $\alpha_T$ agreed within 1% with the experimentally determined value. This offers a rigorous check as to the accuracy of the analyses reported in Table II. Thus, it is possible to analyze ternary mixtures, of which neither the total concentration nor composition is known with an error of approximately 2%.

## Quaternary Systems

Since ether was employed as the solvent for the final extractions of plant tissues, it was selected as the solvent for quaternary systems. The absorption spectra of chlorophylls *A* and *B* as determined by Zscheile (1934) [4] were employed as standards. For leaf xanthophyll and beta carotene the respective absorption spectra were measured in diethyl ether from which the absorption curves (Figure 1) were calculated. In ether both of these pigments have an alpha of 218.5 at λ 4775. The alphas at λ 4850 and λ 4900 are 195 and 158.5 respectively for beta carotene and 139.5 and 78.0 respectively for leaf xanthophyll.

As described for the ternary mixture, aliquot portions were taken from the stock solutions of chlorophylls *A* and *B*. Leaf xanthophyll and beta carotene and mixtures of known composition were made. Spectrophotometric analyses of these mixtures show that the following analytical procedure is feasible:

(1) Total chlorophyll may be determined by employing λ 6450 A.U. with a ± 2.5% error. The absorption coefficients for both the chlorophylls are identical at this wave length.

(2) The error was 1% or less when chlorophyll *A* concentration was determined by λs 6600 and 6700 A.U. Proper analytical checks were possible in determining this component by employing two or more different wave lengths.

(3) Figure 1 illustrates the relative absorption by the plant pigments per 100 parts of chlorophyll *A*. This distribution is for extracts of corn and barley tissues. Figure 1 also illustrates that λ 4775 A.U. permits the measurement of total carotenoids; yet at this wave length the absorption by the chlorophylls is low—only 5 to 6%. The magnitude of the correction may be calculated after total chlorophyll is determined by λ 6450 A.U. Wave lengths 4850 and 4900 A.U. were employed for the analyses of beta carotene (error ± 2.0%) by use of equation 2.

$$\alpha_T = (\alpha_1 - \alpha_2)\frac{C_1}{C_T} + \alpha_2. \qquad (2)$$

[4] On a sample of chlorophyll with the proper magnesium analysis, the author measured the absorption coefficients and found the spectra to be similar to those reported by Zscheile (1934a).

Additional data are presented under analyses of unknown quaternary mixtures, on the validity of employing λs 4775, 4850, and 4900 A.U. in measuring beta carotene and leaf xanthophyll (total xanthophyll).

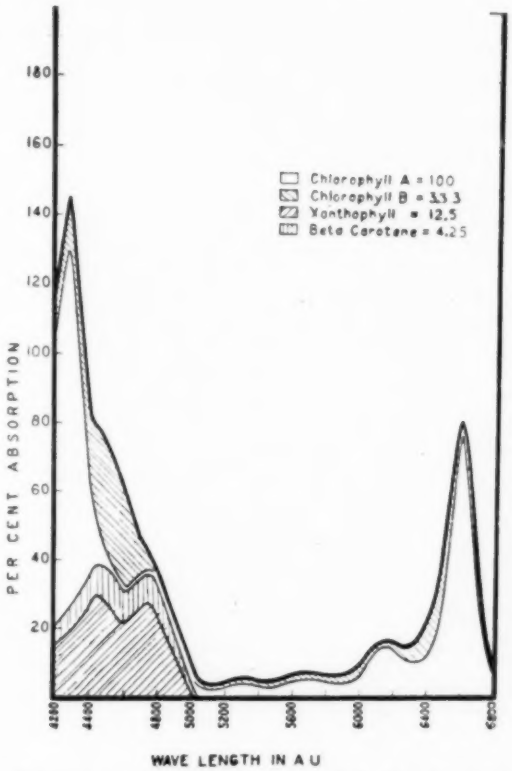


Figure 1. The relative absorption of light by total leaf xanthophyll, beta carotene, and chlorophyll *B* per 100 parts of chlorophyll *A*.

(4) Since total chlorophyll as measured by λ 6450 A.U. contains ± 2.5% error, it becomes 8 to 15% error in chlorophyll *B* analyses (by difference) because the concentration of chlorophyll *B* is so low. Thus, the large band of chlorophyll *B* at λ 4525 A.U. was employed for analysis of this component. Data in Figure 1 show that the absorption by the carotenoids at λ 4525 A.U. is approximately 8 ± 0.5% more than at λ 4775 A.U. Hence, by subtracting the carotenoid absorption at λ 4525 A.U. from $\alpha_T$ 4525 A.U. the difference is due to chlorophyll *B* absorption. This proved possible on the *known solutions* and the error in the chlorophyll *B* analyses was reduced to ± 0.7% (or less).

Briefly, the analyses of quaternary systems may be performed by employing λ 4525 A.U. for chlorophyll *B*, λs 6600 and 6700 A.U. for chlorophyll *A* and λ6450 A.U. for estimating total chlorophyll. Wave

lengths 4775, and 4850 and 4900 A.U., were used for total carotenoids and beta carotene respectively (with proper corrections).

### Unknown Quaternary Mixtures

The above method of analyses has been further standardized to permit the quantitative measurement of chlorophylls *A* and *B*, leaf xanthophyll and beta carotene in barley and corn tissue extracts (in ether). The absorption by the carotenoids at λ 4525 A.U. was compared to that at λs 4700, 4775, 4780, 4790, and 4800 A.U. These measurements were made on saponified extracts of barley and corn tissue. These data are summarized in Table III.

TABLE III

THE RATIO BETWEEN $\beta_T$[b] FOR WAVE LENGTH 4525 A.U. AND $\beta_T$ FOR WAVE LENGTHS 4770, 4775, 4780, 4790 AND 4800 A.U. FOR TOTAL CAROTENOIDS IN ETHER AFTER SAPONIFICATION

| Series | A.U. | $\beta_T$ for λ 4525 A.U. $\beta_{T_1}\cdots$ | $\cdots\beta_{T_5}$ |
|---|---|---|---|
| | Corn tissue extract | | |
| $\beta_{T_1}$ | 4770 | 1.145 | |
| $\beta_{T_2}$ | 4775 | 1.16 | |
| $\beta_{T_3}$ | 4780 | 1.18 | |
| $\beta_{T_4}$ | 4790 | 1.22 | |
| $\beta_{T_5}$ | 4800 | 1.25 | |
| | Barley tissue extract | | |
| $\beta_{T_1}$ | 4770 | 1.07 | |
| $\beta_{T_2}$ | 4775 | 1.09 | |
| $\beta_{T_3}$ | 4780 | 1.106 | |
| $\beta_{T_4}$ | 4790 | 1.13 | |
| $\beta_{T_5}$ | 4800 | 1.16 | |

[b] $\beta_T = \alpha_T C_T$.

The same ratios for $\lambda_1\cdots\ \cdots\lambda_5$ were obtained on tissue extracts of etiolated corn and barley as reported in Table III for mature plants. The ratio for λ4775 A.U. for barley tissue agrees with the ratio presented data in Figure 1; but the ratios for corn tissue extracts are higher, due to the presence of different xanthophyll isomers. Analyses made on corn tissue extracts before and after the removal by saponification [6] are summarized in Table IV.

Data presented in Table IV illustrate that by correcting for total chlorophylls it is possible to determine accurately the total carotenoid concentration in the presence of the chlorophylls.

A similar test was made for beta carotene analyses. When the chlorophylls and total xanthophyll were removed (by saponification and 92% methanol-ligroin partition respectively) the amount of beta

[6] A short saponification was employed. The time factor was 2 minutes. The saponification was carried out by adding the hot MeOH–KOH solution to the extract with the minimum amount of shaking. Moore (12) has shown that long saponification and vigorous shaking may cause chemical changes and oxidation.

carotene present (in 20% diethyl ether and 80% ethanol) agreed with the amount as determined by λ 4850 A.U. in the quaternary mixture. The maximum variation was 2.5 to 3.5% between the two methods for a series of five samples. The error is greatest for the beta carotene component because of its low concentration.

TABLE IV

A Comparison of Total Carotenoid Analyses Before and After the Removal of the Chlorophyll (by Saponification)

| Series | Total carotenoids analyzed in ether in the presence of chlorophylls *A* and *B* | Total carotenoids analyzed after saponification of the chlorophylls in 20% ether and 80% ethanol |
|---|---|---|
| | % | % |
| 1 | 0.07630 | 0.07572 ± 0.00071 |
| 2 | 0.01515 | 0.01483 ± 0.00015 |
| 3 | 0.01385 | 0.01405 ± 0.00024 |
| 4 | 0.01495 | 0.01470 ± 0.00015 |
| 5 | 0.01310 | 0.01336 ± 0.00013 |
| 6 | 0.01305 | 0.01331 ± 0.00011 |
| 7 | 0.01105 | 0.01093 ± 0.00010 |
| 8 | 0.00860 | 0.00872 ± 0.00009 |

The large error (3 to 4%) for beta carotene is unavoidable because it (in leaf tissue extracts) represents only 3 to 4% of the total pigment content. The error in the determination of chlorophyll *A* and leaf xanthophyll is about 1.0%, and 2% or less for chlorophyll *B*.

Burr and Miller (1937) in following the development of the chlorophylls in young etiolated barley and corn, obtain chlorophyll *A*/chlorophyll *B* ratios as large as 100. Under approximately the same experimental conditions Inman (1937) has obtained similar chlorophyll ratios.

## Summary

1. Photoelectric spectrophotometry has been satisfactorily employed to analyze ternary and quaternary mixtures with less than 2% error, providing each component is present in sufficient concentration to form more than 10% of the total pigment content.

2. Unknown ternary mixtures obtained from tomato tissue may be analyzed satisfactorily. By employing two or more wave lengths per sample per component, lycopene, leaf xanthophyll, and beta carotene analyses on duplicate samples agree with an error of only 2%. Part of this error is due to the heterogeneous distribution of the pigments in the tomato tissue.

3. Chlorophyll ratios determined by this physical method confirm those reported by Inman, who employed a chemical method.

4. The method permits ternary and quaternary mixtures to be analyzed with almost the same degree of rapidity as those solutions containing single component pigment.

5. Quantitative analyses can be made with the minimum amount of photo-oxidation—less than the experimental error.

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# THE COLLOIDAL BEHAVIOR OF FLOUR DOUGHS.

## II. A STUDY OF THE EFFECTS OF VARYING THE FLOUR-WATER RATIO [1]

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The term absorption has been used in several different ways in measuring and describing the properties of flour. Not uncommonly it implies the ratio of water to flour required to bring the dough to a definite and more or less arbitrary level of relative plasticity or viscosity. In a limited number of cases it is estimated from the observed plasticity of dough prepared with a definite or constant ratio of flour to water. The authors prefer to regard absorption as the proportion of water to flour which results in dough, and hence in bread, of optimum properties. Thus cognizance is taken of possible changes in dough properties during fermentation. At best, the absorption thus defined is not an absolute value but will vary with any particular lot of flour, depending upon the dough formula, fermentation program, and type of bread desired.

A variety of methods has been employed for determining flour absorption, the details of which were conditioned by the individual's definition of absorption, and the mechanical facilities available. Before testing instruments were employed, the relative mobility of dough was observed by the feeling, or force exerted by the hand in effecting a plastic flow of the dough. Bakers became very skillful in judging doughs by the sense of touch. Then simple viscosimeters made their appearance in a number of forms, that described by Jago and Jago (1911) being typical. In the instance of this instrument, a quantity of dough was placed in a cylinder provided with a standard aperture at the bottom. A unit force was applied by means of a weight at the top of the dough column, and the rate of flow through the plastometer opening was measured.

Briggs' (1918) "Perfekdo" device was somewhat similar. This employed atmospheric pressure, applied by moving a standard glass tube up to the dough surface, reducing the pressure at the other end of the tube by a suction pump and observing the rate of flow of the

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dough into the graduated tube. An elaboration of this general method, but refined to permit of calculating the results in absolute units, was employed by St. John and Bailey (1929).

Rejto's ductility machine, described by Kosutany (1907), enabled him to measure the relative force required to extend doughs containing varying proportions of water. In a series of doughs prepared from the same flour but with varying proportions of water, ranging from 45 to 50% of the flour, the "Zugkraft" was determined and recorded (Kosutany, p. 262) as follows:

| Water, % | Zugkraft, g. |
|---|---|
| 45 | 937.75 |
| 46 | 852.50 |
| 47 | 775.00 |
| 48 | 676.73 |
| 49 | 596.75 |
| 50 | 530.10 |

If the logarithms of water used, and of Zugkraft be plotted, the points fall nearly on a straight line.

Harrell (1927) designed a penetrometer for use with large doughs in the bakery while van der Lee (1929) employed the same general principle in a laboratory instrument.

Chopin's (1921) extensimeter afforded a measure of the force required to extend a dough surface, which was recorded by a pressure gauge. He observed that this force was related to the proportion of water in the dough. Bailey and LeVesconte (1924) investigated the applicability of this device to American flours. Several similar devices including the Buhler "Comparator" (see Kozmin 1936) and the Borasio and de Rege Pneumodynamometer (1934) could doubtless be employed in like manner.

Swanson and Working (1933), and Working (1934) reported on the use of a centrifuge in measuring water absorption and Fifield (1933) described his experiences with the same procedure. Halton and Scott Blair (1936) departed rather widely from the earlier practices; they added water to the point where the dough possessed a standard stickiness as measured by the force required to overcome the adhesion of a metal weight to the surface of the dough.

Another and singularly useful criterion of relative dough mobility is afforded by devices which measure or record the force required to move the arms or blades of a mixing machine through a dough. This method was employed by Bailey (1930) in measuring the work-input (in watt hours) of a dough mixer, the blades of which were rotated a unit number of times. St. John and Bailey (1929) applied this method to the measurement of the influence of added dry skim milk on the absorption of doughs.

Skovholt and Bailey (1932) found that the farinograph recorded the force applied to the blades of a dough mixer, and used this instrument in studying the increase in absorption effected by dry skim milk. Near and Sullivan (1935) observed that dough consistency as measured with the farinograph was highly correlated with the baker's absorption in a series of flours ranging from 9.3 to 15.6% protein.

## Experimental

For the purpose of determining the effects of varying the flour-water ratio five flours were selected, a strong spring wheat straight (No. 16245), a spring wheat clear (No. 15474), a Minnesota winter wheat straight (No. 16246), a family blend patent (No. 16049), and a

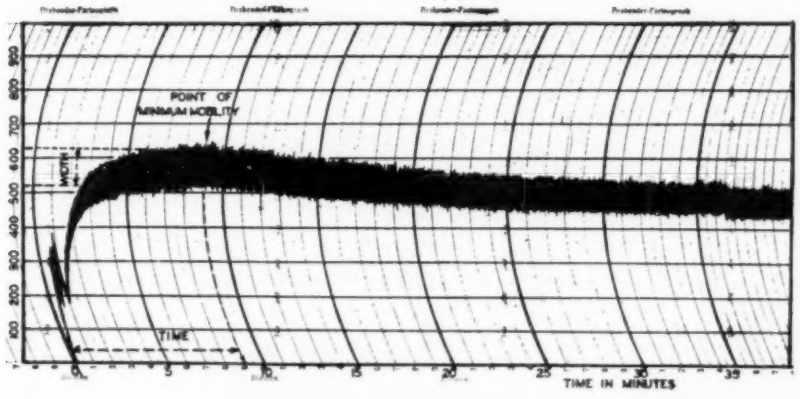


Figure 1. A typical farinograph curve drawn by the pen of the instrument during the mixing of a spring wheat flour dough, exhibiting the methods of measurement of the factors used in this paper.

soft cake patent (No. 15964). Doughs prepared with these flours were mixed in the farinograph with varying amounts of water. The curves drawn by the pen of the instrument were measured for the magnitude of the power-input at the time of minimum mobility, and for the time required to bring the doughs to this point of minimum mobility. The width of the line at the point of minimum mobility was also measured. The method of making these measurements is shown in Figure 1 which represents a typical curve drawn during the mixing of a strong spring wheat flour. The data secured from these flours are presented in Table I.

If the farinograph really measures the viscosity, or its reciprocal the mobility, of doughs then the viscosity with different ratios of flour to water should follow the rule of Sharp and Gortner (1923) for flour suspensions in water. By this rule there should be a curvilinear relation between the water-flour ratio and the viscosity or mobility.

TABLE I

PLASTIC PROPERTIES OF FLOUR-WATER DOUGHS MIXED IN THE BRABENDER FARINOGRAPH

*Flour No. 16246, Minnesota Winter Wheat Straight, 12.00% Protein*

| Absorption | Point of minimum mobility, B. units | Time to point of minimum mobility | Width of line at point of minimum mobility, B. units | Concentration of flour per 100 grams water | Log. conc. flour | Log. point of minimum mobility | Log. time to point of minimum mobility |
|---|---|---|---|---|---|---|---|
| % | | *Min.* | | *G.* | | | |
| 51.5 | 880 | 3.0 | 210 | 194 | 2.288 | 2.945 | .477 |
| 52 | 825 | 3.5 | 140 | 192 | 2.283 | 2.916 | .544 |
| 53 | 750 | 4.2 | 140 | 189 | 2.276 | 2.875 | .623 |
| 54 | 700 | 4.2 | 115 | 185 | 2.267 | 2.845 | .623 |
| 55 | 695 | 7.0 | 115 | 182 | 2.260 | 2.842 | .845 |
| 56 | 595 | 5.5 | 110 | 179 | 2.253 | 2.775 | .740 |
| 57 | 575 | 7.5 | 95 | 175 | 2.243 | 2.760 | .875 |
| 58 | 515 | 7.0 | 95 | 172 | 2.236 | 2.712 | .845 |
| 59 | 490 | 7.5 | 85 | 169 | 2.228 | 2.690 | .875 |
| 60 | 490 | 7.7 | 85 | 167 | 2.223 | 2.690 | .887 |
| 62 | 445 | 9.0 | 85 | 162 | 2.210 | 2.648 | .954 |
| 64 | 390 | 15.5 | 50 | 156 | 2.193 | 2.591 | 1.190 |
| 68 | 325 | 15.0 | 60 | 147 | 2.167 | 2.512 | 1.176 |
| 75 | 250 | 30.0 | 40 | 133 | 2.124 | 2.398 | 1.477 |
| | | | *Flour No. 16245, Spring Wheat Straight, 14.3% Protein* | | | | |
| 60 | 810 | 5.5 | 145 | 167 | 2.223 | 2.909 | .740 |
| 61 | 740 | 6.0 | 130 | 164 | 2.215 | 2.869 | .778 |
| 62 | 735 | 6.0 | 125 | 161 | 2.207 | 2.866 | .778 |
| 63 | 735 | 8.0 | 110 | 159 | 2.201 | 2.866 | .903 |
| 64 | 665 | 6.0 | 110 | 156 | 2.193 | 2.823 | .699 |
| 65 | 655 | 7.0 | 100 | 154 | 2.188 | 2.816 | .845 |
| 67 | 530 | 11.0 | 80 | 149 | 2.173 | 2.724 | .964 |
| 69 | 515 | 13.5 | 80 | 145 | 2.161 | 2.712 | 1.130 |
| 71 | 490 | 14.2 | 75 | 141 | 2.149 | 2.690 | 1.152 |
| 73 | 410 | 22.5 | 60 | 137 | 2.137 | 2.613 | 1.352 |
| 75 | 430 | 28.5 | 60 | 133 | 2.124 | 2.634 | 1.455 |
| 85 | 300 | 41.0 | 45 | 118 | 2.072 | 2.477 | 1.613 |
| | | | *Flour No. 15474, Spring Wheat Clear, 13.5% Protein* | | | | |
| 59 | 855 | 4.5 | 175 | 170 | 2.230 | 2.932 | .653 |
| 60 | 775 | 6.5 | 160 | 167 | 2.223 | 2.889 | .813 |
| 61 | 750 | 7.0 | 160 | 164 | 2.215 | 2.875 | .845 |
| 62 | 730 | 7.7 | 155 | 161 | 2.207 | 2.863 | .887 |
| 64 | 615 | 10.7 | 110 | 156 | 2.193 | 2.788 | 1.029 |
| 66 | 565 | 13.7 | 110 | 151 | 2.179 | 2.752 | 1.137 |
| 68 | 510 | 12.7 | 90 | 147 | 2.167 | 2.708 | 1.104 |
| 70 | 485 | 17.2 | 85 | 143 | 2.155 | 2.686 | 1.236 |
| 72 | 440 | 20.7 | 75 | 139 | 2.143 | 2.644 | 1.316 |
| 74 | 440 | 21.7 | 75 | 135 | 2.130 | 2.644 | 1.336 |
| 78 | 385 | 29.5 | 75 | 128 | 2.107 | 2.586 | 1.470 |

TABLE I—*Continued*

| Absorption | Point of minimum mobility, B. units | Time to point of minimum mobility | Width of line at point of minimum mobility, B. units | Concentration of flour per 100 grams water | Log. conc. flour | Log. point of minimum mobility | Log. time to point of minimum mobility |
|---|---|---|---|---|---|---|---|
| *Flour No. 16049, Family Patent, 11.0% Protein* | | | | | | | |
| % | | *Min.* | | *G.* | | | |
| 54 | 870 | 2.5 | 190 | 185 | 2.267 | 2.940 | .398 |
| 55 | 775 | 5.0 | 135 | 182 | 2.260 | 2.889 | .699 |
| 56 | 700 | 5.0 | 130 | 178 | 2.250 | 2.845 | .699 |
| 58 | 635 | 6.2 | 100 | 172 | 2.236 | 2.802 | .792 |
| 60 | 555 | 6.2 | 85 | 167 | 2.223 | 2.744 | .792 |
| 60 | 560 | 7.0 | 85 | 167 | 2.223 | 2.748 | .845 |
| 62 | 555 | 7.2 | 90 | 161 | 2.207 | 2.744 | .857 |
| 62 | 510 | 14.2 | 70 | 161 | 2.207 | 2.708 | 1.152 |
| 64 | 445 | 8.2 | 65 | 156 | 2.193 | 2.648 | .914 |
| 66 | 440 | 13.7 | 65 | 151 | 2.179 | 2.644 | 1.137 |
| 68 | 380 | 15.5 | 55 | 147 | 2.167 | 2.580 | 1.190 |
| 70 | 385 | 13.2 | 55 | 143 | 2.155 | 2.586 | 1.121 |
| 74 | 310 | 20.0 | 55 | 135 | 2.130 | 2.491 | 1.301 |
| 80 | 270 | 27.0 | 40 | 125 | 2.097 | 2.431 | 1.431 |
| *Flour No. 15964, Soft Cake Patent, 7.9% Protein* | | | | | | | |
| 48 | 930 | 1.2 | 200 | 208 | 2.318 | 2.969 | .079 |
| 52 | 580 | 1.7 | 100 | 192 | 2.283 | 2.763 | .230 |
| 55 | 450 | 2.5 | 65 | 182 | 2.260 | 2.653 | .398 |
| 58 | 375 | 4.5 | 50 | 172 | 2.236 | 2.574 | .653 |
| 62 | 300 | 7.5 | 35 | 161 | 2.207 | 2.477 | .875 |
| 67 | 225 | 9.5 | 30 | 149 | 2.173 | 2.352 | .978 |

In Figure 2 this relation for four of the five flours is graphically recorded. When the difference in manner of expression of the data is taken into consideration it can readily be seen that these curves are essentially similar to those of Sharp and Gortner. Sharp and Gortner working on flour suspensions further found that if the logarithm of the flour concentration was plotted against the logarithm of the viscosity the result was a straight line, having a slope which was a function of the properties of the individual flours.

Our data were treated in like manner, with the results recorded in Figure 3, and it is evident that there is a close approximation to the fitted straight lines. In general it appears that the farinograph measures the viscosity or mobility of such doughs fairly well, at least within the range of viscosity here represented.

The relation between the absorption and the time required to bring the doughs to the point of minimum mobility is presented in

Figure 4. This relation is definitely curvilinear, but may be straightened by plotting the logarithm of the time required to reach the point of minimum mobility against the flour concentration in the dough expressed as grams of flour per 100 g. of water, as has been done in Figure 5. These data are rather surprising in that there appears to be

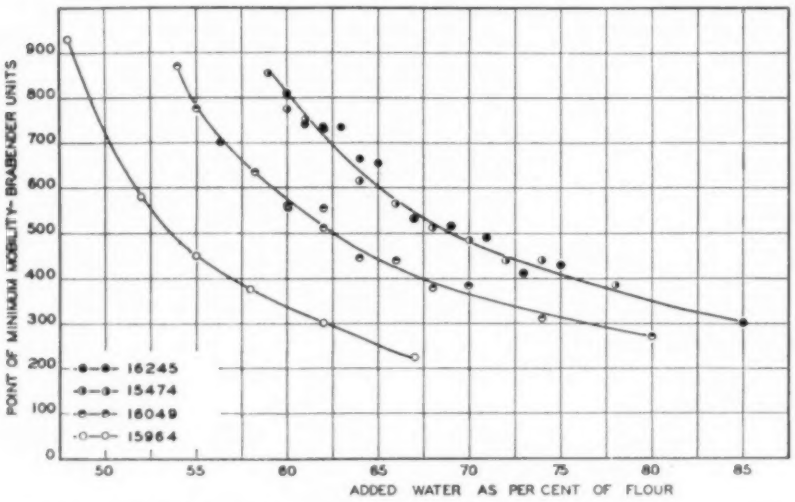


Figure 2. Relation between the amount of water used to form the dough, expressed as percent of the flour, and the minimum mobility of the dough for four typical flours.

no differentiation between the flours, all of which, high-protein spring clear or low-protein cake patent, fall quite well into the linear agreement. This indicates that a high correlation exists between the time required to develop a dough and the amount of water used, even though the mobility at the time of greatest development may vary through wide limits. This relation should receive consideration in developing experimental baking techniques, as it suggests the necessity of departing from a fixed mixing technique with variable amounts of water as specified in the method of the American Association of Cereal Chemists.

The width of the line drawn by the instrument at constant dashpot setting was once suggested as a measure of the elasticity of the dough. The starch-water curves presented in the preceding section of this study (Markley 1937) display quite broad lines, yet it would be absurd to assign any appreciable amount of either tensile or shearing elasticity to a starch-water paste; such a paste may have volume elasticity, but the farinograph is not designed for the measurement of volume elasticity. In Figure 6 is shown the relation between the width of the line and the mobility of the dough at the points of mini-

mum mobility. There is no differentiation of the flours and the relation is definitely curvilinear. It would appear from this that the width of the line drawn by the farinograph pen is essentially a function of the mobility of the dough. It is possible from a theoretical aspect that rigidity may enter into this property of width of line in addition to

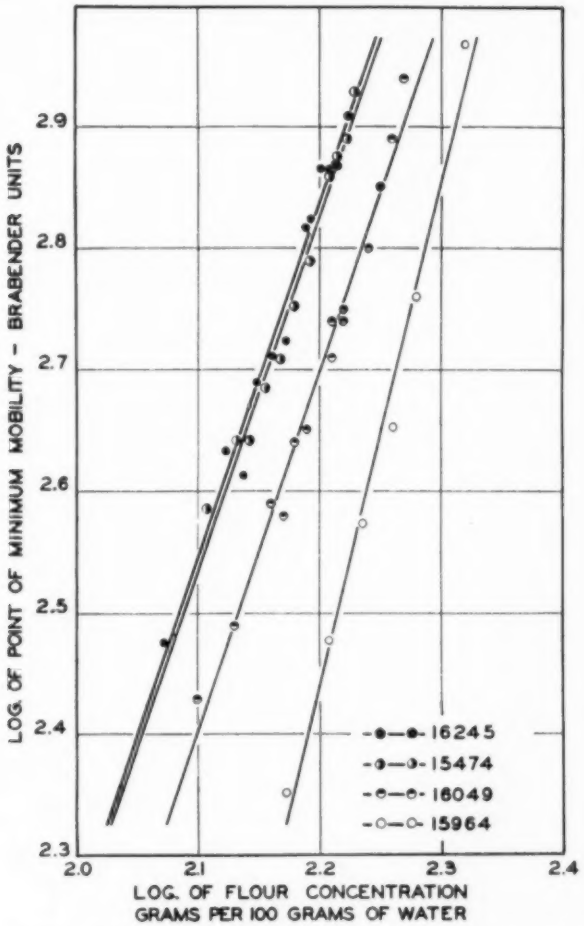


Figure 3. Relation between the flour concentration in the dough, expressed as logarithms of grams of flour per 100 grams of water, and the logarithm of the minimum mobility of the dough in Brabender units for 4 typical flours.

mobility, but as yet we have no precise methods for the measurement of rigidity in doughs. It is quite certain that the width of the line is not a measure of elasticity.

A certain amount of judgment has to be exercised in translating into baking practice the absorption values as determined by the farinograph. Certain flours, as has been shown by Near and Sullivan

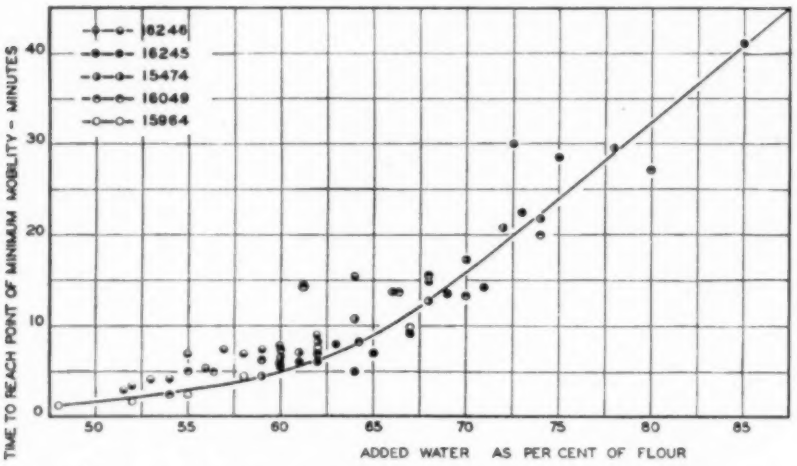


Figure 4. Relation between the amount of water used in forming the dough and the time required to bring the dough to the condition of minimum mobility for 5 typical flours.

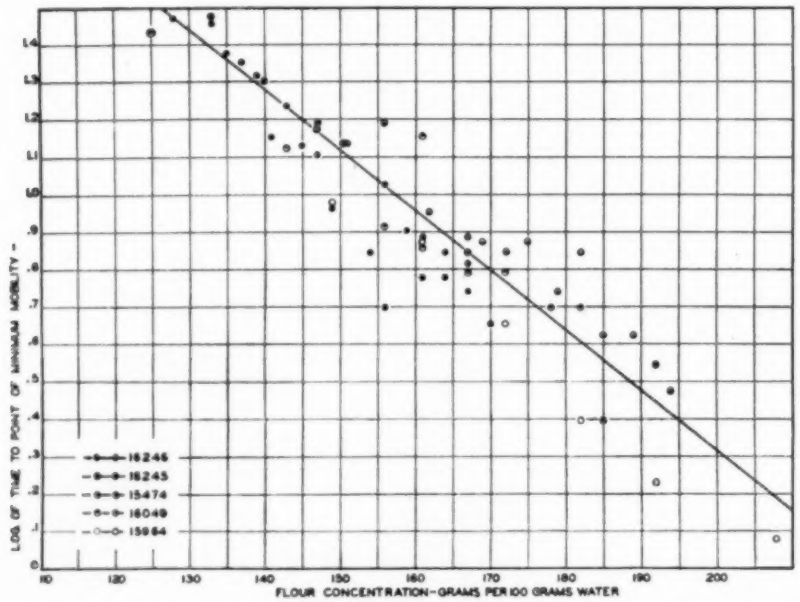


Figure 5. Relation between the flour concentration in the dough, expressed as grams of flour per 100 grams of water, and the logarithm of time required to bring the dough to its condition of minimum mobility for 5 typical flours.

(1935), which tend to slack-off during fermentation must be mixed with less water than the recording dough mixer indicates. This is not a fault of the instrument, but merely means that even though a dough is correct in plastic properties upon leaving the mixer, it may not be in an equally good condition at the conclusion of the fermentation period.

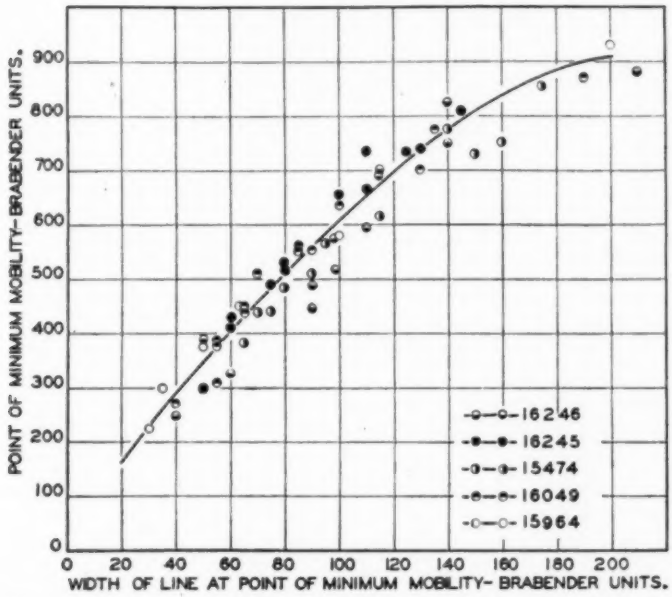


Figure 6. Relation between the width of the line drawn by the pen of the farinograph with the mobility readings in Brabender units at the point of minimum mobility for 5 typical flours.

## Summary

Water required to bring doughs to definite minimum mobility can be measured by a recording dough mixer.

Absorption and viscosity, or mobility, in doughs are similar to flour concentration and viscosity in the more dilute flour-water systems studied by Sharp and Gortner with viscosimetric technique.

Absorption and time required to mix a dough are closely related in a curvilinear fashion regardless of the protein content of the individual flours. This line can be straightened by an appropriate mathematical process.

Width of the line drawn by the farinograph pen is a function of the mobility of the dough and not of its elasticity.

## Acknowledgments

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# THE EFFECT OF THE SUBSTRATE ON DIASTATIC ACTIVITY

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## Introduction

Not much attention has been paid to the variability of the soluble starch used for diastatic power determinations; and it is not sufficiently recognized that soluble starches prepared by different workers or manufacturers give different results when used with the same amylase preparations. Lampé (1922) found a difference of 11 to 16% in the diastatic power of a given malt using the soluble starches of Kahlbaum, Merck, Riedel, and Schering. He used the Windisch-Kolbach method and his results were also checked by Windisch. Silbernagel (1930) also showed that the Lintner value of a malt sample depended on the particular lot of soluble starch used in the determination. He suggested the use of starch paste instead of soluble starch, but this is not generally suitable. Recently, Kauert (1937) found that the soluble starch exerted the greatest influence in the determination of diastatic power.

The malt analysis committee of the American Society of Brewing Chemists (1935) in drawing up specifications for the method of determining diastatic power probably also recognized this same difficulty because they laid down very careful specifications for the starch to be used. However, the effect of deviations from these specifications is not stated, and therefore a sample of starch might be rejected and yet satisfy a more fundamental test of its suitability as given later.

Snider and Coleman (1937) have discussed the suitability of starch as a substrate for amylase in terms of its reducing power, pH, erythrodextrin content, and Lintner value of a standard malt They concluded, among other things, that a low phosphate (or low ash) content is desirable for optimum diastatic activity; that the reducing substances in soluble starch should not exceed 1% in terms of maltose; and that soluble starch solutions before addition of buffer should have a pH of 4.6.

Sallans and Anderson (1937) found that the standard error for starches is of the order of $\pm$ 3%.

It became necessary while developing a new method for determining diastatic power by application of the principles given by Johnston and Jozsa (1935) to set up a standard for soluble starch which could be duplicated without comparison with a previous batch of starch. The most descriptive method of studying the influence of the soluble starch on diastatic action is by a study of the kinetics of the reaction. Hanes (1932) has already pointed out the advantages of this method. In this manner by maintaining all variables—pH, temperature, quantity of enzyme, starch, concentration of substrate—constant except the starch, the effect of the starch is revealed. The rate curve method of study demands an extended experimental procedure, but it gives the most complete and reliable information of any method.

## Experimental

### Reagents

The ferricyanide method of sugar analysis is probably the most convenient method for estimating the reducing substances formed by diastasis and was therefore chosen for this work. The following reagents are required:

*Standard sodium thiosulfate solution:* Dissolve 12.41 g. of $Na_2S_2O_3 \cdot 5H_2O$ in distilled water which has been previously boiled and cooled and dilute to one liter. Standardize this solution against the 0.1 *N* $KIO_3$ solution by pipetting 12.50 ml. of the $KIO_3$ solution into an Erlenmeyer flask, adding 5 ml. of 50% KI and 4 ml. of 6 *N* HCl (in the order named). Titrate immediately with the thiosulfate using 3 ml. of starch indicator toward the end of the titration. The solution is preserved by the addition of a few drops of $CS_2$.

*Standard potassium iodate solution:* Dissolve 3.5672 g. of $KIO_3$ in distilled water and dilute to one liter. The concentration of this solution is exactly 0.1 *N*.

*Alkaline ferricyanide solution:* Dissolve 16.5 g. of C.P. $K_3Fe(CN)_6$ and 22 g. of $Na_2CO_3$ in distilled water and dilute to one liter. This solution must be protected from light. Standardize it against the thiosulfate solution by pipetting 25.00 ml. of the ferricyanide solution into a 300 ml. Erlenmeyer flask, adding 25 ml. of the $ZnSO_4$ reagent, then 5 ml. of 50% KI, and titrating immediately with the thiosulfate. If the solution is not exactly 0.05 *N*, adjust it to this strength.

*Zinc sulfate reagent:* Dissolve 50 g. of $ZnSO_4 \cdot 7H_2O$ and 100 g. of KCl in 600 ml. of distilled water. Add 200 ml. of glacial acetic acid and dilute to one liter. Crystals of $K_2SO_4 \cdot ZnSO_4 \cdot 6H_2O$ will gradually settle out on standing, but this will not harm the solution.

*Starch indicator solution:* Make a 1% solution of soluble starch in water. Preserve by the addition of a few drops of toluene, which is just as satisfactory as NaCl and more convenient to use.

*Acetate buffer solution:* To 1.10 mols of acetic acid is added sufficient approximately 4 *N* carbonate-free NaOH solution so that when the mixture is diluted to one liter the pH will be 5.00. About 0.73 mols of NaOH will be necessary. The total acetate concentration of this buffer solution is 1.10 molar and is so calculated that when 50 ml. of it are used for each liter of starch solution and 100 ml. of starch solution are mixed with 10 ml. of malt solution, the final buffer concentration will be 0.05 molar. This concentration is sufficient to insure maximum activation of the amylase according to the work of Sherman, Caldwell, and Boynton (1930).

*2 N NaOH and HCl solutions:* It is not necessary that these solutions be exactly 2 *N*, but they should be equivalent, *i.e.*, 10 ml. of the NaOH should just neutralize 10 ml. of the HCl.

*Starch solution:* Mix 20 g., based on dry weight, of soluble starch with a small quantity of distilled water and pour while stirring into 750 ml. of boiling distilled water. Continue boiling for 2 to 3 minutes after the starch is added. Then cool the starch solution and rinse into a one-liter volumetric flask. Add 50 ml. of acetate buffer and dilute to volume. The water content of the starch is readily determined by drying 5 g. of it for one hour at 120° C. in a forced-draft drying oven.

*Malt solution:* Exactly 5 g. of a highly active (approximately 261° L.) malt syrup are weighed out in an aluminum weighing scoop and transferred to a 500 ml. volumetric flask and made to volume with distilled water. The malt syrup is kept in a glass-stoppered bottle and stored in the ice box at 3.3° C. No loss in activity occurred during the time of these experiments. The malt syrup is used in preference to dry malt because of the convenience in making up a fresh solution each day.

## Procedure for Determining Rate Curves

Hanes (1932) described a very satisfactory procedure for studying the early stages of the saccharification of soluble starch by malt amylase. The following procedure, based on Hanes, is used to determine the rate curves.

The reaction is carried out in 300 ml. Erlenmeyer flasks supported in a large water thermostat maintained at 25.00 ± 0.05° C. All pipets used are protected with cotton plugs to exclude traces of saliva. United States Bureau of Standards calibrated pipets are always used except for adding the starch solution.

Before a run is started, the solutions to be used are allowed to come to the temperature of the water bath; and 50 ml. portions of the alkaline ferricyanide solution are pipetted into 300 ml. Erlenmeyer flasks from an automatic pipet.

Ten milliliters of the malt solution are pipetted into an Erlenmeyer flask. After waiting a few minutes to insure temperature equilibrium, 100 ml. of the starch solution are added from a fast (30-second) pipet noting the time with a stopwatch. About one minute before the time the first sample is to be taken, a clean, dry pipet is filled with the reaction mixture and allowed to drain back into the flask. Then 30 seconds before the desired time, the pipet is refilled and the level adjusted to the mark. The tip is then freed from adhering drops and inserted into one of the flasks containing alkaline ferricyanide. At the desired time—2, 4, 6, etc., minutes—the sample is delivered into the ferricyanide. The delivery is hastened by blowing out most of the liquid, allowing, however, the last drops to drain out in the time specified on the pipet, while holding the tip of the pipet against the side of the flask. The flask is then gently rotated to wash down the sides. The procedure for subsequent samples is the same. A 10 ml. pipet is used for the 2, 4, 6, 8 and 10-minute samples, and a 5 ml. pipet for the 15, 20, and 30-minute samples.

Hanes (1932) showed that his alkaline ferricyanide reagent will kill the amylase. By direct measurement, the pH of the alkaline ferricyanide used here is found to be approximately 12. Ohlsson (1930) found that at 38° C. and pH > 10.5 both alpha- and beta-amylase are completely killed, which shows that the alkalinity of the ferricyanide is sufficient to arrest diastatic action completely.

The blank is determined by pipetting 100 ml. of starch solution into a 200 ml. volumetric flask, adding 10 ml. of 2 *N* NaOH, 10 ml. of malt solution, and 10 ml. of 2 *N* HCl, in the order named. The solution is made to volume, and a 25 ml. aliquot is taken for analysis. The initial reducing power is determined on a 25 ml. sample of the original starch solution. The same result is obtained with or without buffer salts present.

### Determination of Reducing Substances

The reducing substances formed in the starch, in the blank mixture, and those formed by the amylase are then estimated by a slight alteration of Gore and Steele's (1935) modification of the Blish and Sandstedt (1933) ferricyanide method.

Sufficient distilled water is added to the ferricyanide-sugar solution to make a total volume of 75 ml. The flasks are then heated in a boiling water bath for exactly 20 minutes and quickly placed in the

thermostat at 25°. After the flasks have cooled for at least five minutes and not more than one hour, 50 ml. of the $ZnSO_4$ reagent and 5 ml. of the KI solution are added and the solution immediately titrated with the standard thiosulfate.

The flasks are supported during the boiling on a false bottom made of ⅜-inch mesh galvanized wire screen which insures uniform heating. Since the oxidation of sugars by ferricyanide is an incomplete reaction, all determinations must be carried out in exactly the same manner. Uniform cooling of the flasks in a thermostat gives more consistent results than cooling in tap water. In this laboratory tap water varies from 7° in the winter to 21° C. in the summer. The oxidized sugar solutions must not be allowed to stand after adding the $ZnSO_4$ reagent because, as shown by Putnam, Blish, and Sandstedt (1935), an increase in maltose values occurs.

The pH of the 2% starch solutions, without buffer, is measured with a Hellige glass electrode calibrated against 0.05 molar acid potassium phthalate. The amount of erythrodextrin is estimated qualitatively by Snider and Coleman's (1937) modification of Small's (1919) method.

## Maltose Standardization

The relation of the milliliters of 0.05 *N* ferricyanide consumed to maltose oxidized was determined against pure recrystallized maltose hydrate. The specific rotation of the sample used was $[\alpha]_0^{22.6°} = +130.8°$. Varying quantities of this maltose were placed into 300 ml. Erlenmeyer flasks, 50 ml. of ferricyanide added and the oxidation carried out as previously described. From these results it is found that the relation between milliliters of ferricyanide and milligrams of maltose hydrate is linear as far as 84 mg. From these data the equation of the best straight line was derived by the method of least squares and is $M = 1.791\ F$ ($M < 84$ mg.),

where $M$ = number of milligrams of maltose hydrate

and $F$ = number of milliliters of 0.05 *N* ferricyanide.

Using this relation it is easy to calculate the amount of maltose formed in each interval of time. All results are corrected by subtraction of the blank, part of which is due to the malt syrup and part to the starch.

## Soluble Starches Used in the Study

Thirteen soluble starches were used. Ten of them were of commercial grade and three were prepared in the laboratory. Two of the laboratory preparations were prepared, after Lintner (1908), by

covering BKMF potato starch with 7.5% HCl, and allowing it to stand for 7 days at approximately 18° C. The starch was then filtered off, washed with distilled water, the remaining acid neutralized with $NaHCO_3$ (using neutral "lacmus" paper as indicator), and finally washed free from chloride. It was then air dried and ground. The other starch was prepared by Gore's (1928) method. It will be noticed that all of these starches are not Lintner starches. The other starches were included in order to compare them with the Lintner starches which have been accepted generally as a standard in the study of diastatic power. Soluble starches prepared by the Lintner method are not especially unique, but the method is easy, inexpensive, not too drastic, and gives a starch relatively free from impurities. A description of these starches will be found in Table I.

TABLE I

DESCRIPTION OF THE STARCHES

| Soluble starch number | Nature | Origin | Labeled |
|---|---|---|---|
| 1 | Laboratory preparation | Potato | Lintner |
| 2 | " " | " | " |
| 3 | Commercial | " | " |
| 4 | " | " | " |
| 5 | " | " | " |
| 6 | " | " | " |
| 7 | " | " | Improved Lintner |
| 8 | " | " | |
| 9 | " | " | Indicator |
| 10 | " | " | Practical |
| 11 | " | " | C.P. |
| 12 | " | " | C.P. |
| 13 | Laboratory preparation | " | |

## Discussion of Rate Curves

A rate curve was run for each of these starches. Each curve is the average of two experiments. The average deviation from the mean is not more than 2% and in most cases is about 1%. This seems to be the precision attainable by this method. Figure 1 shows the rate curves for four of these starches which are characteristic of all. It can be seen that all of these curves are linear for approximately the first ten minutes, and the rate at zero time is easily determined from the large-scale graph on which these curves were constructed. The data from which these curves were constructed as well as those for the remainder of the rate curves given in this paper are conveniently assembled in Table II.

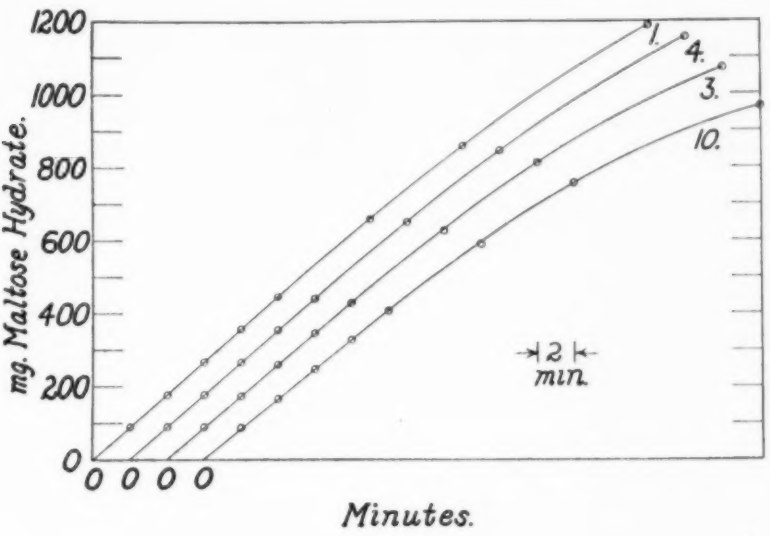


Figure 1

TABLE II

DATA FOR ALL RATE CURVES

| Time, minutes / Starch number | 2 | 4 | 6 | 8 | 10 | 15 | 20 | 30 |
|---|---|---|---|---|---|---|---|---|
| | Mg. of maltose hydrate (average values) | | | | | | | |
| 1 | 89 | 177 | 267 | 357 | 446 | 658 | 859 | 1189 |
| 2 | 91 | 178 | 266 | 354 | 441 | 647 | 849 | 1182 |
| 3 | 88 | 171 | 258 | 343 | 427 | 626 | 810 | 1071 |
| 4 | 89 | 177 | 265 | 353 | 440 | 649 | 846 | 1156 |
| 5 | 92 | 179 | 269 | 357 | 445 | 650 | 852 | 1170 |
| 6 | 91 | 174 | 264 | 352 | 441 | 644 | 843 | 1161 |
| 7 | 90 | 178 | 268 | 355 | 437 | 645 | 837 | 1121 |
| 8 | 90 | 178 | 265 | 354 | 439 | 642 | 837 | 1119 |
| 9 | 86 | 168 | 250 | 332 | 412 | 601 | 775 | 1015 |
| 10 | 86 | 166 | 246 | 327 | 406 | 589 | 757 | 965 |
| 11 | 92 | 179 | 270 | 360 | 450 | 660 | 863 | 1182 |
| 12 | 89 | 178 | 267 | 356 | 444 | 648 | 849 | 1179 |
| 13 | 93 | 181 | 273 | 362 | 453 | 672 | 887 | 1236 |
| 3F | 92 | 178 | 266 | 355 | 439 | 640 | 832 | 1109 |
| 4F | 89 | 176 | 265 | 354 | 439 | 648 | 849 | 1170 |
| 9F | 88 | 174 | 259 | 344 | 426 | 627 | 817 | 1097 |
| 10F | 87 | 171 | 254 | 337 | 418 | 613 | 790 | 1036 |
| 50% 4F 50% 10F | 92 | 177 | 265 | 352 | 435 | 636 | 828 | 1116 |
| 25% 4F 75% 10F | 87 | 175 | 258 | 344 | 424 | 618 | 802 | 1069 |

Table III gives the moisture content, initial rate, reducing power, and maltose formed in 30 minutes. By giving the amount of maltose formed in 30 minutes along with the initial rate, the curve is defined more completely.

TABLE III

CHARACTERISTICS OF THE SOLUBLE STARCHES
*(Arranged in order of increasing initial rate)*

| Starch number | Initial rate, Mg. maltose[1] per minute | R.P. % maltose[1] | pH | Erythro-dextrin | Mg. maltose[1] in 30 min. | Moisture % |
|---|---|---|---|---|---|---|
| 10 | 41.1 | 11.31 | 3.50 | ++++ | 965 | 10.37 |
| 9 | 41.7 | 11.74 | 3.40 | ++++ | 1015 | 12.09 |
| 3 | 42.9 | 5.47 | 3.55 | ++++ | 1078 | 18.22 |
| 6 | 44.2 | 1.96 | 5.05 | + | 1161 | 13.31 |
| 8 | 44.2 | 2.65 | 5.60 | ++++ | 1119 | 17.38 |
| 4 | 44.3 | 3.06 | 3.83 | ++ | 1156 | 11.04 |
| 7 | 44.3 | 4.15 | 4.20 | +++ | 1121 | 8.04 |
| 12 | 44.4 | 1.26 | 6.00 | ++ | 1179 | 14.64 |
| 2 | 44.5 | 1.57 | 5.85 | + | 1182 | 12.88 |
| 1 | 44.6 | 1.93 | 6.45 | + | 1189 | 7.64 |
| 5 | 44.8 | 1.97 | 4.96 | + | 1170 | 13.77 |
| 11 | 44.9 | 3.33 | 5.90 | +++ | 1182 | 12.18 |
| 13 | 45.4 | 10.05 | | ++++ | 1236 | 9.38 |

[1] Maltose in this paper actually means maltose hydrate.

A study of Figure 1 and Table III reveals several facts. First, we can note the approximate exactitude of Kjeldahl's law if we use it in its modern form which is given by Ling (1936) as follows: "When malt extract is allowed to act on starch paste—at the present time soluble potato starch solution is used—and the saccharification measured by the reducing power of the conversion liquid, expressed in terms of maltose, the saccharifying power is a linear function of the time or mass, provided the reducing power expressed as percent of maltose on the dry starch does not exceed 30%." In the present case this amounts to 630 mg. of maltose hydrate. The rate curves in all cases are only strictly linear for ten minutes or to approximately 450 mg. of maltose; the deviation from linearity at 630 mg. is small, but nevertheless distinct.

A second important conclusion is that there is no correlation between reducing power and initial rate. This is contrary to what had been expected in view of the importance attached to it by the American Society of Brewing Chemists. As can be seen, values of R.P. from 1.26% to 4.15% give practically the same initial rate. A low initial R.P., however, is desirable because, as both Clark (1912) and Hanes (1935) have pointed out, we cannot correct exactly for the initial R.P. without information concerning the nature of the reducing substances and their fate during diastasis. The authors feel, nevertheless, that since it is impossible to get a soluble starch without any initial R.P.—even raw starch paste has 0.15% R.P.—more uniform results are obtained if the blank is used. Our results indicate that a blank correction is necessary in order to attain a precision of $\pm$ 2%.

As is easily seen there is also no relation between the pH of the unbuffered starch solution and the initial rate. Variations from pH 3.83 to 6.45 show practically the same initial rate. This result is surely to be expected. However, Snider and Coleman attempted to show that "the problem with respect to the acidity of soluble starch . . . is not, however, its adjustment to a pH of 4.5 to 4.7 by means of buffers, but rather which starch is more desirable—the one close to the starch neutral point (pH = 4.6) on the acid side, or the one close to the starch neutral point on the alkaline side." The activity of malt amylase is determined with respect to pH only by the pH of the substrate solution when buffered and not upon its previous pH history.

Snider and Coleman also concluded that a low phosphate content, indicated by a low ash content, of soluble starch is desirable for optimum diastatic activity. The authors could not conclude this from Snider and Coleman's data, and furthermore do not consider it of importance when acetates are present in a large quantity as they are in the Lintner determination. Sherman, Caldwell, and Boynton (1930) showed that malt amylase exhibited the same activity in starch solutions containing the same concentration, 0.01 *M*, of acetate and phosphate provided the systems had been adjusted to their respective pH-optima. The pH-optima for acetate and phosphate differ by approximately 0.1 to 0.2 pH units and in the case of the Lintner determination must be conditioned only by the large excess of acetate ion present.

The relative quantity of erythrodextrin does not seem to affect the initial rate as can be easily seen, and therefore is not as critical a factor as suggested by Snider and Coleman.

Certain aspects of the data of Table III demand further consideration. Starch No. 3 is a Lintner starch and yet it shows a lower initial rate than the rest of the Lintner starches. Starch No. 13 has a high R.P. and despite this has the highest initial rate of any starch studied, quite in contrast with starches Nos. 9 and 10 which have about the same R.P. but very low initial rates. Lüers and Wasmund (1922) found that maltose inhibits diastasis, and Ohlsson (1930) found that dextrins are saccharified more easily than either soluble starch or starch paste. These two papers suggested that perhaps starch No. 13 consists largely of dextrins and very little reducing sugars, whereas starches Nos. 3, 9, and 10 contained more reducing sugars which tend to slow down the reaction.

### Determination of Fermentable Sugars

In order to determine the quantity of reducing substances present, whether maltose, glucose, or perhaps low dextrins, the biological method of Schultz and Kirby (1933) was selected as suitable. No attempt was made to differentiate different sugars, but all substances fermentable by baker's yeast are calculated as maltose. The method of analysis slightly modified from Schultz and Kirby is as follows. The apparatus used is exactly the same. One hundred milliliters of water, 1.5 g. of $NH_4H_2PO_4$, 0.6 g. of $(NH_4)_2HPO_4$, 1 g. of maltose, and 3 g. of fresh baker's yeast are shaken together until the initial charge of maltose is completely fermented. The shaking is then stopped, a weighed sample (10 to 20 g.) of the starch is added and shaking resumed until fermentation is complete. By assuming 210 ml. of $CO_2$ to be equivalent to 1 g. of maltose, the percent of fermentable substances calculated as maltose is easily obtained. The large gasometers which were available make the results only approximate, but what is more important the relative magnitude and order are correct. The results are given in Table IV in increasing order of percent fermentable substances and repeating the initial rate and R.P. for comparison.

TABLE IV

QUANTITY OF FERMENTABLE SUBSTANCES IN SOLUBLE STARCHES

| Starch number | Fermentable substances as % maltose | R.P. % maltose | Initial rate, Mg. maltose per minute |
|---|---|---|---|
| 1 | 0.34 | 1.93 | 44.6 |
| 11 | 0.37 | 3.33 | 44.9 |
| 4 | 0.41 | 3.06 | 44.3 |
| 2 | 0.42 | 1.57 | 44.5 |
| 8 | 0.44 | 2.65 | 44.2 |
| 7 | 0.49 | 4.15 | 44.3 |
| 6 | 0.58 | 1.96 | 44.2 |
| 5 | 0.74 | 1.97 | 44.8 |
| 13 | 1.0 | 10.05 | 45.4 |
| 3 | 1.8 | 5.47 | 42.9 |
| 9 | 3.0 | 11.74 | 41.7 |
| 10 | 3.6 | 11.31 | 41.1 |

It can be seen that there is a good agreement between the amount of fermentable substances and the initial rate. Of course it is realized that the initial rate is influenced by more factors than just this one, but in general when the percent of fermentable substances is low, the initial rate is what we may call normal for soluble starches and averages 44.5 mg. of maltose per minute for the standard quantity of malt syrup used in testing. The difference between starches Nos. 3 and

13 is quite apparent. Starch No. 3 has a lower rate than No. 13 although the R.P. of No. 13 is almost twice that of No. 3. Nos. 9 and 10 with a large percentage of fermentable substances have the lowest rates of all. There is no relation at all between fermentable sugars and R.P.

### Kinetics of Fermented Starches

It was now thought that perhaps here is the suggestion for a method of making the rate curves for all these starches coincide if they are equally susceptible to diastase. Several of these starches were then fermented with yeast in the following manner to remove all fermentable substances. Three grams of $NH_4H_2PO_4$, 1.2 g. of $(NH_4)_2HPO_4$, 3 g. of fresh baker's yeast, 150 g. of soluble starch, and 400 ml. of distilled water are mixed together in a one-liter Erlenmeyer flask. The flask is placed in a shaking apparatus and shaken overnight which insures complete fermentation. The starch-yeast mixture is now filtered on a Büchner funnel. The mixture is suspended in distilled water and divided into four parts which are poured into 250-ml. centrifuge bottles. The mixture is centrifuged until the starch is well packed down. Most of the yeast comes to the top and is removed by scraping with a spatula. The starch is suspended again in water and separation repeated. A third time suffices to remove the yeast almost completely. The starch is then filtered again on a Büchner funnel, washed, sucked dry, and dried two days between filter paper. After grinding, the moisture content is determined in the usual manner. The yields varied from 60 to 85% depending on the care used in scraping off the yeast. A yeast count made on one sample gave 7000 cells per gram in the fermented starch as against 400 cells per gram in the original starch. This illustrates the success of this simple method of yeast removal. The very small percent, approximately 0.001% by weight, of yeast remaining does not interfere with the sugar determination because it must be remembered that the starch solution is boiled before use which effectively kills all the yeast cells and their concomitant enzymes.

Starches Nos. 3, 4, 9, and 10 were treated by this fermentation method and rate curves were determined for each. Table V gives the R.P., initial rate, and the amount of maltose formed in 30 minutes compared with the same values on the original starch.

In all four starches the residual R.P. is always less than the difference between the original R.P. and quantity of fermentable sugars, showing definitely that the fermentable substances are not all maltose. The R.P. of starches Nos. 9 and 10 is reduced quite remarkably by fermentation. The initial rate of starch No. 3 has been raised from

TABLE V

CHARACTERISTICS OF THE FERMENTED STARCHES

| Starch number | 3F | 4F | 9F | 10F |
|---|---|---|---|---|
| Prepared from soluble starch number | 3 | 4 | 9 | 10 |
| R.P. % maltose | | | | |
| Before | 5.47 | 3.06 | 11.74 | 11.31 |
| After | 3.16 | 2.31 | 5.42 | 5.27 |
| Initial rate, Mg. maltose/min. | | | | |
| Before | 42.9 | 44.3 | 41.7 | 41.1 |
| After | 44.6 | 44.5 | 43.4 | 42.4 |
| Mg. maltose in 30 min. | | | | |
| Before | 1078 | 1156 | 1015 | 965 |
| After | 1109 | 1170 | 1097 | 1036 |

42.9 to 44.6 mg. of maltose per minute, which is the normal value, but the rate drops off more rapidly after 10 minutes as shown by the 30-minute value, 1109 mg. The average normal value at 30 minutes is 1174 mg. of maltose. Therefore not all of this starch is apparently equally susceptible to diastase. The initial rates of both Nos. 9 and 10 are increased, but not up to the normal value, indicating that these starches are also not quite as susceptible to diastase as a normal starch. Starch No. 4, which is what may be called a normal starch (defined more completely later), is affected very little by this fermentation treatment.

To help decide whether starch No. 10 is less susceptible towards diastase, the rate curves for two mixtures of fermented starches Nos. 4F and 10F were determined, and the results are given in Table VI, which are compared with the same values for the separate starches.

TABLE VI

| Composition of mixture | Initial rate | Maltose in 30 minutes |
|---|---|---|
| 50% 4F<br>50% 10F | 44.0 | 1116 |
| 25% 4F<br>75% 10F | 43.1 | 1069 |
| 4F | 44.5 | 1170 |
| 10F | 42.4 | 1036 |

It will be noted that the initial rate of the 50–50 mixture is 44.0 mg., whereas the corresponding rate for the 25–75 mixture is 43.1 mg. This can be accounted for on a statistical basis. The enzyme molecules acting on the 50–50 mixture make, since we are dealing with a statistical system, more collisions with susceptible starch molecules than when acting on the 25–75 mixture. Accordingly, the effect of

the proportion of susceptible molecules will be reflected in the initial rate. The 44.0 mg. initial rate of the 50–50 mixture is approximately equal to the corresponding rate for starch 4F, showing that for the first few minutes the enzyme molecules make sufficient collisions with susceptible starch molecules to permit maintenance of rate. However, as the proportion of susceptible molecules diminishes the rate falls off rapidly. This is borne out by the 43.1 mg. initial rate of the 25–75 mixture.

The rate-curve study of these fermented starches leads us to believe that some of these starches have been altered in some manner during the process of manufacture so that they differ in their internal structure and cannot be made to act normally without effecting structural changes. We have no explanation for these variations but are planning further work which may clarify the situation.

### Definition of Normal Starch

From the results of all these studies, the soluble starches studied can be separated into two groups as shown in Table VII.

TABLE VII

SACCHARIFICATION INDEX OF SOLUBLE STARCHES

| | Starch number | Initial rate A | Maltose in 30 minutes B | Ratio $\frac{B}{A}$ |
|---|---|---|---|---|
| Group A | 1 | 44.6 | 1189 | 26.6 |
| | 2 | 44.5 | 1182 | 26.6 |
| | 4 | 44.3 | 1156 | 26.1 |
| | 5 | 44.8 | 1170 | 26.1 |
| | 6 | 44.2 | 1161 | 26.3 |
| | 11 | 44.9 | 1182 | 26.4 |
| | 12 | 44.4 | 1179 | 26.5 |
| Group B | 3 | 42.9 | 1078 | 25.2 |
| | 7 | 44.3 | 1121 | 25.4 |
| | 8 | 44.2 | 1119 | 25.3 |
| | 9 | 41.7 | 1015 | 24.4 |
| | 10 | 41.1 | 965 | 23.4 |
| | 13 | 45.4 | 1236 | 27.2 |

Those starches in Group A are what we may call normal. By normal we mean that when used with the same amylase concentration all starches in this group will give the same rate curve within a precision of $\pm$ 2%. Or expressed in another way, the same Lintner value for the enzyme preparation will be obtained when using any starch of Group A as the substrate. All starches in Group B will not give the same rate curve; and may or may not give the same Lintner value. Starches Nos. 7 and 8 would give the same Lintner value as the

starches of Group A because they have a normal initial rate and the Lintner method utilizes only the linear portion of the rate curve. However, their 30-minute conversion points are low and therefore their rate curves do not coincide with the normal rate curves of Group A.

In column 4 of Table VII we have listed the calculated ratios of the amount of maltose formed in 30 minutes to the initial rate for all starches studied. This ratio may be identified as an index to the suitability of a given starch for enzyme concentration measurements. From the data given in Table VII and other data not tabulated in this paper, it is found that a normal starch has a saccharification index from 26.1 to 26.8. Consequently we suggest that a normal starch may be chosen from an unknown group of soluble starches by carrying out a saccharification reaction using sufficient enzyme to hydrolyze the starch at an initial rate from 44 to 45 mg. of maltose hydrate per minute. The enzyme used shall be preferably a high diastatic malt syrup. If a dry malt must be used then it shall be extracted with 5% NaCl solution substituted for the water in the official method. The necessity of using NaCl for the extraction has been proved by repeated tests and will be reported in a future paper. If the saccharification index is within the limits 26.1 to 26.8, then the investigator may conclude that he has a normal starch at hand. It may be added that the starch should also have as low an initial reducing power as possible.

Anderson (1937) points out that "the possibility of differences of 10% between laboratories, occurring merely as a result of differences in the starches cannot be viewed with equanimity." He further states that in his opinion the difficulty can not be adequately overcome by adopting more stringent specifications for the starch because the testing of the starch would be too difficult.

The authors feel that the two specifications presented here are simple enough for any routine laboratory to apply and yet exact information is given concerning the suitability of a given starch.

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# THE MEANING OF FERMENTATION TOLERANCE

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(Read at the Annual Meeting, June 1936)

In considering the subject of fermentation tolerance, information was desired upon what this term meant to cereal workers. A questionnaire was therefore sent out requesting a definition of the term. The author wishes to express his sincere appreciation to the cereal chemists, bakers and bakery engineers named below for their answers to this questionnaire sent them by the writer. The ideas expressed by these workers in their replies have formed the basis for the following discussion on fermentation tolerance.

A. W. Alcock, Winnipeg, Manitoba; Carl L. Alsberg, Stanford University, Calif.; M. J. Blish, Lincoln, Nebr.; Ralph Bohn, Chicago, Ill.; Mary M. Brooke, Omaha, Nebr.; L. E. Caster, Rockford, Ill.; A. E. Curtis, Kansas City, Mo.; Claude F. Davis, Salina, Kans.; R. K. Durham, Kansas City, Mo.; Charles N. Frey, New York, N. Y.; George F. Garnatz, Cincinnati, Ohio; W. F. Geddes, Winnipeg, Manitoba; C. G. Harrel, Minneapolis, Minn.; W. L. Heald, Kansas City, Mo.; L. E. Jackson, Chicago, Ill.; Arnold H. Johnson, Baltimore, Md.; I. O. Juvrud, Chicago, Ill.; R. K. Larmour, Saskatoon, Saskatchewan; L. E. Leatherock, Wichita, Kans.; C. A. McDuffee, Joplin, Mo.; W. J. Ocken, Chicago, Ill.; L. R. Olsen, Minneapolis, Minn.; Peter G. Pirrie, New York, N. Y.; John Roberts, Los Angeles, Calif.; A. A. Schaal, Cambridge, Mass.; B. J. Sullivan, Minneapolis, Minn.; E. F. Tibbling, Kansas City, Mo.; Wm. Walmsley, Chicago, Ill.

There were 28 replies to the questionnaire. Three replies stated that fermentation tolerance of a dough was dependent entirely upon the sugar level or gassing power of the dough; two replies were so general and indefinite that no specific definition was given; while 23 replies expressed very closely the ideas embodied in the following paragraphs.

Three of the 23 replies defined fermentation tolerance so concisely that they are quoted at length as a theme for more detailed consideration.

*Reply No. 1.* "I regard fermentation tolerance as the length of time over which a good loaf of bread (judged from the standpoint of flavor, volume, texture, etc.) can be produced from a given flour dough. The proper balance between gas production and gas retention (the latter factor being dependent on the 'quality' and quantity of gluten) is the most important factor governing the fermentation tolerance of a flour. In my opinion, fermentation tolerance is dependent fully as much on the amount of gluten and its physical characteristics as on the sugar content of the dough."

*Reply No. 2.* "My understanding of fermentation tolerance is

the range of time during which the two main factors of bread baking are in balance, that is, gas production and gas retention, and produce quality bread."

*Reply No. 3.* "To me, the fermentation tolerance always meant the range of time over which the gas production and gas retention could be kept in the proper balance to produce satisfactory bread."

All the reactions and procedures involved in bread making, may be divided into three groups, namely: mixing, fermentation and baking. Under mixing would be found the changes which take place in the dough batch as the various ingredients are brought together and incorporated into a homogeneous mass. Each flour requires a very definite period of time for its optimum mechanical development which varies according to its own varietal characteristics, the temperature of mixing, the kind and proportion of materials used, the speed of the mixer and other conditions imposed. This mixing period is dependent upon or is the resultant of all these forces reacting upon each other simultaneously and the end of the period or the point of optimum dough development is very definitely indicated to the baker by the familiar "clean-up" in the mixer. No stated time can be assigned for a mixing period until this "clean-up" is observed. Following this test a mixing time can be stated provided all the materials and conditions remain constant, a situation which is very difficult to maintain. When the dough "cleans up" in the mixer it indicates that an equilibrium has been reached between all the forces of absorption, ingredients, temperature, mixing speed and other factors. This is the pivotal point of dough development mechanically. The dough should be dumped from the mixer, either before, upon or after the "clean-up" depending upon the flour in question.

Now begins the period of fermentation. It is started with a dough in a balanced condition. If the fermentation period were skipped and the dough baked immediately upon coming from the mixer, the bread would be solid, heavy, hard, flavorless and unpalatable. In order to aerate the dough, make it light and give the bread volume, it is necessary to generate gas within the mass. Two groups of forces now start to operate, namely, those of gas production and of gas retention. Gas in the dough is generated largely by the enzymes diastase, maltase and zymase. The gas retention or colloidal dispersion of the dough is developed by enzymes (principally protease), by chemical means (ash, moisture, H-ion, bleach, oxidizers, etc.) and by some mechanical agencies (stretching under gas formation, punching, dividing, rounding and molding). Therefore, the objective to be accomplished during the fermentation division of the bread making process is to generate sufficient gas for maximum aeration and to disperse properly the colloidal structure of the dough so that a maximum amount of the gas will be retained.

The forces of gas production and of gas retention work simultaneously and integrate each other. Each strives for supremacy. If maximum gas production is reached before the colloidal elasticity of the dough is at an optimum, there is not enough gas left to aerate the mass when the elasticity peak is reached. Again if the dough reaches its maximum elasticity or gas retention peak before there is maximum gas production, much of the gas is lost. There is a peak of gas generation and a peak of gas retention. The ideal adjustment of all forces would cause the gas production peak to be reached in the same length of time from the start of fermentation as the gas retention peak. When both peaks are reached at the same time there frequently is combined in one loaf the largest volume together with the best grain, texture, crust color and other loaf characteristics which the flour in question will produce.

There is usually a range of fermentation time over which the forces of gas production are in balance with the forces of gas retention, during which period good bread or the desired product can be produced. It is reasonable to call this range in fermentation time the fermentation tolerance of the dough. As has been pointed out above, this range is dependent upon the balancing of all the forces of gas production against all the forces of gas retention. It is, therefore, apparent that fermentation tolerance is a resultant of many forces and of many tolerances and is frequently measured in units of time.

If a fermentation time is selected which is shorter than the fermentation tolerance, the bread is inferior. The same is true if a fermentation period is selected which is longer than the fermentation tolerance. Some flours and their doughs when handled in certain ways have very narrow ranges of fermentation tolerance. These same flours and doughs when handled in other ways or under formula adjustments may display a wide range of fermentation tolerance.

The baker strives so to regulate the factors of gas production and gas retention that they will produce their respective peaks simultaneously, thus combining into his bread the largest volume with the best grain, texture and other physical characteristics which the flour can produce. Fermentation is over when the dough enters the oven. Because of this fact the baker not only gains considerable information on the proper adjustment of the gas production and gas retention forces by the action of the dough during pan proof, but he also frequently uses different pan-proof times as a test of the correct fermentation.

Baking is the last step in the bread-making process. Many reactions occur in the oven. The test for the close of this period is a thoroughly baked loaf of bread. The ultimate goal of all three steps in bread production is good bread that measures up to the desired standard.

# VARIATION IN CAROTINOID PIGMENT CONCENTRATION AMONG INBRED AND CROSSBRED STRAINS OF CORN [1]

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(Read at the Annual Meeting, May 1937)

The present-day method of corn improvement based upon the production of relatively homozygous inbred strains by controlled pollination followed by their recombination into hybrids offers exceptional opportunities for studies of variability in chemical composition. The inbred strains thus developed, as well as the hybrids between them, show wide differences in morphological characters. Many studies have been previously reported on the extent of variation in morphological characteristics among inbred strains and their hybrids but few studies have been made on the physiological differences between inbred or crossbred strains of corn.

Since inbred strains show striking differences in intensity of yellow endosperm color, the present study was made to determine the range in concentration of carotinoid pigments in the endosperm and to determine the extent to which variations in endosperm color were associated with variations in carotinoid pigment concentration. Analyses were also made to learn if differences in carotinoid pigment concentration in the endosperm might also be reflected in the leaf tissue of young plants grown under greenhouse conditions.

## Experimental Material and Methods

The study on the variation in carotinoid pigments in corn was made on 19 inbred lines selected to represent a wide range in yellow endosperm color. The lines used had been inbred from 6 to 14 years and were homozygous for their respective color differences. Approximately 10 grams of shelled corn were ground in a Wiley mill, the pigments extracted with acetone and transferred to an ether solution in a separatory funnel.

In the comparison of carotinoid pigments in the endosperm and in young plants, four pairs of lines resulting from crosses of yellow and white endosperm parents were used. . The lines had been maintained

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in a heterozygous condition for yellow endosperm during the inbreeding period, and subsequently purified for yellow and colorless endosperm. Each pair of lines was then relatively similar for all other genetic characters and differed only in endosperm color. Leaf pigment concentration was determined on the young plants grown in the greenhouse when they were 12 to 15 inches tall.

In another experiment, suitable crosses were made to provide endosperm genotypes of *YYY*, *YYy*, *Yyy*, and *yyy*, to study the relation between the number of dominant genes for yellow endosperm and the concentration of carotinoid pigments in the endosperm and in seedling plants.

The concentration of beta carotene,[3] total carotinoids and chlorophyll was determined spectrophotometrically by the method described by Burr and Miller (1937).

## Experimental Results

The data obtained in this study will be presented under three parts, variation in carotinoid pigments in the endosperm of inbred lines of corn, the relation between the number of dominant genes for yellow endosperm color to the concentration of carotinoid pigments, and the relation between carotinoid pigments in the endosperm to that in young corn plants.

### Variation in Carotinoid Pigment Concentration Among Inbred Lines

Among the many yellow endosperm lines produced by inbreeding corn may be found a wide range in color from pale to very dark yellow. To determine the extent to which variations in degree of yellow endosperm color influenced the amount of carotinoid pigments, a group of 19 lines was selected which covered the maximum range available among inbred lines used in the corn improvement program. The seed was grown in 1935 and was fully matured and normal. Among the 19 lines, six were classified as dark yellow, seven medium yellow, and six as light yellow in color. The distribution of the lines in percentage of total carotinoid pigments is given in Table I.

The data given in Table I show that inbred lines may exhibit wide differences in percentage of carotinoid pigments, the 19 lines used in this study ranging from 0.0020 to .00158 in per cent of total carotinoid pigment. From the standpoint of differences in nutritional value, with particular reference to vitamin A, a knowledge of variability in carotene may be of great importance in breeding for improvement in feeding qualities. The wide differences obtained with the limited number of lines used suggest that a more extensive survey and study should be made among inbred strains of yellow corn to determine the possibilities of improvement in this respect.

[3] Beta carotene refers to that fraction soluble in ligroin and insoluble in 92% methanol.

TABLE I

VARIATION IN CAROTINOID PIGMENTS IN THE GRAIN OF 19 INBRED STRAINS OF CORN

| Range in total carotinoid | Number of lines |
|---|---|
| % | |
| .0001 –.00030 | 1 |
| .00031–.00050 | 4 |
| .00051–.00070 | 3 |
| .00071–.00090 | 1 |
| .00091–.00110 | 3 |
| .00111–.00130 | 5 |
| .00131–.00150 | 1 |
| .00151–.00170 | 1 |

The 19 lines were placed in three groups for intensity of endosperm color and the averages for total carotinoid pigments and range of variability determined (see Table II).

TABLE II

RELATION BETWEEN INTENSITY OF YELLOW ENDOSPERM COLOR IN CORN AND THE PERCENTAGE OF TOTAL CAROTINOID PIGMENTS

| Endosperm color | Number of lines | Average percentage total carotinoids | Range |
|---|---|---|---|
| Dark yellow | 6 | .000869 | .000443–.00121 |
| Medium yellow | 7 | .000991 | .000389–.00137 |
| Light yellow | 6 | .000609 | .000202–.00158 |

The lack of a strong relationship between the intensity of yellow endosperm color and the percentage of total carotinoids may be explained in part on the basis that many of the dark and medium yellow endosperm lines also carry genes for brown aleurone. The combined effect of the genes for yellow endosperm with those for brown aleurone usually results in a darker yellow color than those having the genes for yellow endosperm only. The light yellow lines averaged lowest in total carotinoids. These results would indicate some relation between total carotinoids in the endosperm and in endosperm color although dark yellow endosperm lines may not necessarily have a higher percentage of carotinoid pigments than light yellow endosperm lines.

### Relation Between the Number of Dominant Genes for Yellow Endosperm and Carotinoid Pigments in the Endosperm

In feeding studies with homozygous yellow, heterozygous yellow, and homozygous white kernels on ears segregating for endosperm color, Hague and Trost (1928) have shown that vitamin A is transmitted exclusively with yellow endosperm color. No transfer of genes for vitamin A from the yellow parent to white kernels on segregating ears was found since white kernels on segregating ears were not more effec-

tive in promoting growth in rats than the white kernel parent used in the cross. Steinback and Boutwell (1920) had previously shown that white endosperm maize varieties were deficient in vitamin A while yellow endosperm varieties gave normal growth in rats. Yellow kernels selected from variegated maize gave better growth than white or pale yellow kernels.

From data published by Mangelsdorf and Fraps (1931) it was shown that the amount of vitamin A in corn was nearly directly proportional to the number of dominant genes for yellow endosperm color.

Suitable pollinations were available by which the four possible classes of endosperm genotypes *YYY*, *YYy*, *Yyy*, and *yyy* could be analyzed for beta carotene and total carotinoid pigments. The young plants from the four endosperm genotypes were grown in the greenhouse and analyzed for total carotinoids and for total chlorophyll. The data obtained in this study are given in Table III.

TABLE III

RELATION BETWEEN THE NUMBER OF DOMINANT GENES FOR YELLOW ENDOSPERM IN CORN TO THE CONCENTRATION OF CAROTINOID PIGMENTS IN THE ENDOSPERM AND IN THE LEAF TISSUE OF YOUNG PLANTS

| Endosperm genotype | Carotinoid pigments in the endosperm | | | | Total carotinoids in young plants | Total chlorophyll in young plants |
|---|---|---|---|---|---|---|
| | Total carotinoids | | Beta carotene | | | |
| | % | *Ratio* | % | *Ratio* | % | % |
| YYY[1] | .000465 | 3.3 | .000131 | 3.1 | .01206 | .0990 |
| YYy | .000282 | 2.0 | .000079 | 1.9 | .01105 | .0677 |
| Yyy | .000139 | 1.0 | .000042 | 1.0 | .01305 | .0713 |
| yyy | .000042 | 0.3 | .000011 | 0.3 | .00600 | .0487 |

[1] Average of two strains.

The data given in Table III show that the percentage of total carotinoids and beta carotene is approximately in direct proportion to the number of dominant *Y* genes. The ratios of 3.3, 2.0, and 1.0 for total carotinoid and 3.1, 1.9, and 1.0 for beta carotene correspond very closely with 3, 2, and 1 dominant *Y* genes. Colorless endosperm kernels contain only a very small amount of carotene. These results obtained by a physical method of analysis agree very closely with the results obtained from feeding experiments reported by Mangelsdorf and Fraps (1931).

### Relation Between Carotinoid Pigment Concentration in the Endosperm to Carotinoid Pigment Concentration in Young Plants

The data in Table III suggested that seedlings grown from homozygous yellow endosperm kernels were not greatly different in con-

centration of carotinoid pigments than those grown from kernels heterozygous for yellow endosperm. The seedlings from the homozygous yellow kernels contained .01206% total carotinoids and the seedlings from heterozygous kernels .01205% total carotinoids (average of the two classes of heterozygous endosperms). The percentage of total carotinoids in the young plants from homozygous white kernels was found to be approximately one-half as great as from the yellow kernels. To study this relationship further, the seedlings from four pairs of yellow and white endosperm sister lines were analyzed for total carotinoids. These four pairs of inbred lines were maintained in a heterozygous condition for endosperm color during the period of inbreeding. Since the separation into homozygous yellow and white endosperm was not made until the lines were practically homozygous, these sister lines should be nearly identical for other genes. In this group of lines the percentage of carotinoid pigments in the grain was not determined, since previous analysis (see Table III) had shown that white endosperm lines were very low in carotinoid pigments. The average percentage of carotinoid pigments and total chlorophyll for the four white and four yellow sister lines are given in Table IV.

TABLE IV

RELATION BETWEEN ENDOSPERM COLOR IN CORN AND THE CONCENTRATION OF CAROTINOID PIGMENTS AND TOTAL CHLOROPHYLL IN YOUNG PLANTS

| Endosperm | Pigment concentration in young corn plants | | |
|---|---|---|---|
| | Total carotinoids | Beta carotene | Total chlorophyll |
| | % | % | % |
| Yellow | .0083 | .00277 | .0845 |
| White | .0118 | .00361 | .1134 |

The results given in Table IV indicate clearly that there is no apparent relation between the formation of carotinoid pigments in the endosperm and the development of carotinoid pigments in young plant tissue. Although the amount of carotinoids in the plants grown from colorless endosperm kernels is somewhat higher than from yellow endosperm kernels, these differences may be due in part to the small numbers of comparisons used in this study.

These results also show that the genotype which does not permit formation of any appreciable quantities of carotinoids in the endosperm has no inhibiting effect in the formation of carotinoids in young plant tissue. From the standpoint of nutritional value of young corn plants, with respect to vitamin A, it is evident that yellow and white corn varieties are of equal value.

From the data given in Table IV, there appears to be some indica-

tion of a positive relationship between the amount of chlorophyll and the amount of carotinoid pigments in corn leaves. The data in Table III also indicate that the concentration of these two groups of pigments is closely associated.

Unpublished data obtained by Burr and Miller[4] show that when such physiological factors as light intensity and quality of light are varied, the total carotinoid concentration fluctuates with the total chlorophyll concentration.

The extent to which these two pigment classes are associated in mature corn plants is being studied at the present time and will be reported at a later date.

## Summary

The mature grain from 19 inbred lines of corn varying from light to dark yellow endosperm color showed wide differences in percentage of total carotinoid pigments. The variation in pigment concentration was not closely associated with the intensity of yellow endosperm color.

A very close relationship was obtained between the number of dominant *Y* genes for yellow endosperm color and concentration of total carotinoids and beta carotene. The percentage concentration obtained was in direct proportion to the number of dominant genes.

Young corn plants from white and yellow endosperm sister lines were analyzed for chlorophyll and carotinoid pigments. The percentage of carotinoid pigments in leaf tissue from white endosperm lines was found to be slightly higher than from their yellow endosperm sister lines. The differences may be due to the small number of samples used. These results suggest that the formation of carotinoid pigments in the leaf tissue and that deposited in the endosperm are independent of each other.

A positive association was obtained between the percentage of chlorophyll and carotinoid pigments in corn leaf tissue.

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# THE AMYLASES OF DIFFERENT BARLEY VARIETIES[1]

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(Read at the Annual Meeting, May 1937)

Barley has maintained its position as an important cereal crop in no small measure because of its malting properties. It is well suited to the production of malt because the endosperm is covered with a husk which protects the growing acrospire and which also serves as a filtering medium during the brewing process. Furthermore, malted barley is high in diastatic activity and contains less undesirable protein fractions than other malted cereals.

Notwithstanding the interest in the problem of the enzymatic changes involved in malting, there have not been many studies reported concerning the diastatic activity of different American barley varieties. Dickson and coworkers (1935) made a comparative study of malting barleys grown in Wisconsin. They found that Manchuria, Velvet, and Oderbrucker yielded better quality malts than any of the other varieties studied. These varieties were also highest in diastatic activity.

Shellenberger and Bailey (1936a) made an extensive study of the more common barley varieties grown in Minnesota. They observed that Oderbrucker and Velvet were high and Peatland was low in diastatic activity. They also measured the dextrinizing ($\alpha$-amylase) activity of the ungerminated barley, the green malt, and the dry malt of each variety. Their data indicated that ungerminated barley may contain a small but measurable amount of $\alpha$-amylase activity.

Various other studies have presented conflicting conclusions as to the actual existence or absence of $\alpha$-amylase in dormant mature cereals. Nordh and Ohlsson (1932) stated that $\alpha$-amylase was entirely absent in dormant barley and that the diastase of the ungerminated kernels was pure $\beta$-amylase. The findings of Lüers and Rümmler (1933) and Chrzaszcz and Janicki (1933, 1936) supported Ohlsson's conclusion.

The work of Jozsa and Gore (1932) and of Gore and Jozsa (1932) indicated that $\alpha$-amylase, as well as $\beta$-amylase, is present in normal wheat flour. Sandstedt, Blish, Mecham, and Bode (1937) state that

[1] Paper No. 1552, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Claude H. Hills to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

"the widespread belief that ungerminated grain contains only beta amylase, in contrast to germinated or malted grain, which contains both the alpha and beta forms, may be an erroneous one."

## Materials and Methods

The barley used in this study was furnished by the Agronomy Division of the University of Minnesota from the increase plots at University Farm, St. Paul, Minnesota. The following eight varieties were obtained from the 1935 crop: Oderbrucker, Velvet, Trebi, Peatland, Glabron, Wis. No. 38, Spartan, and Minn. No. 462 (an unnamed variety). These last two varieties were not grown in the 1936 crop, so they were replaced by Odessa and Manchuria.

The samples from these two crop years afforded excellent material for making seasonal comparisons. The growing season of 1936 was extremely hot and dry and produced barley having small, shriveled kernels, high in protein content and diastatic activity. The season of 1935 was quite the reverse. Nearly optimum conditions prevailed during the growing season, resulting in barley having large, plump kernels that were high in starch and low in protein content.

*Malting procedure:* The barley samples were malted in the laboratory malting equipment designed and described by Shellenberger and Bailey (1936). Eight 300-gram samples of barley were placed in cheese-cloth bags and steeped in a large jar of water at 12.5° C. to 13.0° C. for 52 hours. Air was bubbled through the steep continuously and the temperature of the steep was controlled by a continuous stream of cold water entering the jar.

At the end of the steeping period the samples of barley were transferred to wire germinating cages and placed in the previously cooled and humidified malting cabinet. The barley was allowed to germinate at 16° ± .5° C. until the acrospires of the grain attained the length of the kernel. This required approximately six days. A constant stream of humidified air was drawn through the cabinet and the cages were revolved one-quarter turn every four hours to insure uniform conditions of germination. On completion of germination the malt was dried inside the malting cabinet according to the following schedule: 12 hours at 30° C., 12 hours at 35° C., 12 hours at 40° C., 12 hours at 50° C., and 6 hours at 70° C.

*Extraction of amylases:* The fresh green malt was taken from the malting cabinet at the desired stage of germination and immediately stored in paper bags in a room maintained at —20° C. The frozen samples were ground through a small hand-driven food chopper operated in the refrigerated room. The dry malt samples, after having

the sprouts removed, were ground through two series of small rolls having 28 and 40 corrugations per inch respectively. Ungerminated barley was ground on the same set of rolls and to the same degree of fineness.

Water extracts of dry malt and ungerminated barley for the determination of diastatic activity were prepared as follows: 2.5 g. (dry basis) of ground barley or malt were extracted with 50 c.c. of water for 3 hours at 21° C., shaking the flask every 30 minutes. Then the extraction mixture was poured into a centrifuge tube and centrifuged at half speed for exactly 5 minutes.

Green malt extracts were more difficult to prepare. In order to insure adequate extraction of enzymes, it was necessary to grind the chopped green malt to the same degree of fineness as the ground barley and dry malt samples. This was accomplished by triturating 2.5 g. (dry basis) of chopped frozen green malt with 5 g. of clean crushed quartz and a little water in a mortar. The finely ground sample was washed into a flask with additional water to make a total of 50 c.c. of water added. The conditions of extraction were the same as for the barley and dry malt.

*Extraction of "total" amylase:* Water extracts of barley and malt were used to determine the "free" or water-soluble amylases. The extraction of "total" amylases was effected by partially digesting the sample with papain. To 50 c.c. of the water extraction mixture were added 250 mg. (.5%) of papain and the sample digested 24 hours at 21° C. The mixture was centrifuged and the supernatant liquid used for the determination of "total" $\alpha$- and $\beta$-amylases. Hills and Bailey (1938) have shown that the "total" amylase activity of barley determined in this manner is equal to the sum of the water-soluble amylase and the amylase activity of the water-insoluble residue.

*$\beta$-Amylase:* All $\beta$-amylase determinations were made in the following manner: To 35 c.c. of 2% soluble starch solution containing 0.1 N acetate buffer (pH = 4.65) was added a sufficient volume of 0.1 N acetate buffer (pH = 4.65) to make the final volume (substrate + enzyme addition) equal 46 c.c. Then 2 c.c. of a 1:10 dilution of the original barley or malt extract were added and the mixture digested 1 hour at 30° ± .1° C. At the end of 1 hour enzyme action was arrested by the addition of 2 c.c. of 10% $H_2SO_4$ and 2 c.c. of 12% $Na_2WO_4$. The quantity of maltose produced was determined on a 5 c.c. aliquot by Blish and Sandstedt's (1933) modification of the Hagedorn and Jensen (1923) micro method for determining blood sugar.

*$\alpha$-Amylase:* Alpha amylase activity was determined by a modification of the Wohlgemuth (1908) method. Progressively increasing

amounts of the barley or malt extract, suitably diluted, were added to a series of test tubes in an ice-water bath. Each tube contained 1 c.c. of 2% soluble starch buffered at pH = 4.65 with 0.1 N acetate buffer. Water was added to make the total volume equal 6 c.c., and the mixture digested 1 hour at 40° C. ± .1° C. The tubes were removed, placed in an ice-water bath and 10 c.c. of ice-cold water and 1 drop of 0.1 N iodine solution were added to each tube. The end-point was the hue possessed by the tube, observed by artificial light, which most nearly matched the RV Normal tone of Chart D in Mulliken's book (1911). The number of cubic centimeters of 2% starch solution used in this instance (1 c.c.) divided by the fraction of a cubic centimeter of the original enzyme solution required, gives the Wohlgemuth value for the change of starch iodine color from blue to violet. This value accordingly expresses the equivalent number of cubic centimeters of 2% soluble starch solution dextrinized by 1 c.c. of the original enzyme extract in 1 hour at 40° C.

TABLE I

FREE AND TOTAL AMYLASE ACTIVITY OF UNGERMINATED BARLEY. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

| Laboratory number | Variety | Dry weight basis: 1000-kernel weight G. | Dry weight basis: Protein N × 6.25 % | Amylase activity: Free (Mg. maltose) | Amylase activity: Total (Mg. maltose) | Ratio A[1] |
|---|---|---|---|---|---|---|
| | | 1935 Crop | | | | |
| 209 | Oderbrucker | 26.36 | 15.94 | 130 | 261 | 2.01 |
| 210 | Velvet | 26.17 | 16.44 | 132 | 257 | 1.95 |
| 211 | Trebi | 33.55 | 16.13 | 141 | 287 | 2.04 |
| 212 | Peatland | 23.17 | 17.69 | 83 | 285 | 3.43 |
| 213 | Glabron | 27.40 | 16.94 | 115 | 239 | 2.08 |
| 214 | Wis. No. 38 | 27.73 | 16.19 | 112 | 210 | 1.88 |
| 215 | Spartan | 38.36 | 16.31 | 90 | 175 | 1.94 |
| 216 | Minn. No. 462 | 24.73 | 15.81 | 96 | 185 | 1.93 |
| | Average | 28.43 | 16.43 | 112 | 237 | 2.16 |
| | | 1936 Crop | | | | |
| 201 | Oderbrucker | 14.55 | 20.00 | 168 | 359 | 2.14 |
| 202 | Velvet | 15.17 | 20.25 | 150 | 342 | 2.28 |
| 203 | Trebi | 18.80 | 22.81 | 225 | 485 | 2.16 |
| 204 | Peatland | 12.40 | 21.63 | 112 | 406 | 3.63 |
| 205 | Glabron | 18.30 | 19.56 | 152 | 303 | 1.99 |
| 206 | Wis. No. 38 | 15.58 | 19.81 | 115 | 253 | 2.20 |
| 207 | Odessa | 14.63 | 21.13 | 157 | 373 | 2.38 |
| 208 | Manchuria | 14.01 | 20.88 | 162 | 430 | 2.65 |
| | Average | 15.43 | 20.76 | 155 | 369 | 2.43 |

[1] Ratio A = $\frac{\text{Total amylase activity of barley}}{\text{Free amylase activity of barley}}$.

### Free and Total Amylase Activity of Barley and Malt

It was desirable to measure not only the water-soluble amylases, but also the total amylase activity of each of the barley samples. Table I gives the free and total amylase activity of the 16 barley samples studied and the ratio between these two values. The protein content and 1,000-kernel weight are also given in order to compare these data with the amylase activity of each variety.

The diastatic activity of the 1936 samples is much greater than for the previous year's crop. There is also a large variation in protein content and 1,000-kernel weight for the same varieties from different crop years. The barley samples grown in 1936 are higher in protein content and lower in 1,000-kernel weight than the same varieties from the 1935 crop.

The varieties Peatland and Trebi were consistently high in total amylase for both years. Manchuria, Velvet, and Oderbrucker were also high in diastatic activity. Wis. No. 38, Spartan, and Minn. No. 462 are relatively low in diastatic activity. There are no consistent varietal differences in protein content for both years.

The increase in amylase activity by papain extraction is indicated in column 7 as the ratio between total and free amylase activity. In general, this increase ranges from 100% to 130%. Peatland shows an abnormally high proportion of bound amylase. There is very little correlation between protein content or 1,000-kernel weight and the ratio of total to free amylase activity.

The amylase activity of green malt and dry malt is recorded in Table II.

The varietal and seasonal differences observed in amylase activity of ungerminated barley appear also in the green malt and dry malt. There is excellent agreement between the total amylase activity of barley before and after malting as evidenced by the constancy of Ratio B. The correlation between total amylase activity of ungerminated barley and of green malt is highly significant ($r = +0.991 \pm 0.003$). This would indicate that the total $\beta$-amylase activity of ungerminated barley is an accurate criterion for predicting the total $\beta$-amylase activity of malted barley. The increase in total $\beta$-amylase activity on malting was 25% to 29%. Myrbäck and Myrbäck (1936) observed a 30% increase in total amylase on germination of barley.

Ratio C indicates that the per cent of total malt amylase of green malt that is water-soluble is 66% and 75% respectively for the two crop years. The increase in the proportion of water-soluble amylase during germination is undoubtedly due to the action of proteolytic enzymes. There is a slight decrease of approximately 16% to 19% in total $\beta$-amy-

TABLE II

AMYLASE ACTIVITY OF GREEN MALT AND DRY MALT. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

| Laboratory number | Variety | Amylase activity of green malt | | Ratio B [1] | Ratio C [2] | Amylase activity of dry malt | | Ratio D [3] |
|---|---|---|---|---|---|---|---|---|
| | | Free | Total | | | Free | Total | |
| | | Mg. maltose | | | | Mg. maltose | | |
| | | 1935 Crop | | | | | | |
| 209 | Oderbrucker | 263 | 358 | 1.37 | .73 | 248 | 302 | 1.19 |
| 210 | Velvet | 218 | 320 | 1.25 | .68 | 220 | 285 | 1.12 |
| 211 | Trebi | 238 | 368 | 1.28 | .65 | 240 | 320 | 1.15 |
| 212 | Peatland | 220 | 355 | 1.25 | .62 | 230 | 327 | 1.09 |
| 213 | Glabron | 187 | 276 | 1.15 | .68 | 185 | 225 | 1.23 |
| 214 | Wis. No. 38 | 163 | 265 | 1.26 | .62 | 182 | 228 | 1.16 |
| 215 | Spartan | 125 | 210 | 1.20 | .60 | 140 | 178 | 1.18 |
| 216 | Minn. No. 462 | 155 | 233 | 1.26 | .67 | 187 | 203 | 1.15 |
| | Average | 196 | 296 | 1.253 | .656 | 204 | 259 | 1.16 |
| | | 1936 Crop | | | | | | |
| 201 | Oderbrucker | 381 | 457 | 1.27 | .83 | 328 | 388 | 1.18 |
| 202 | Velvet | 363 | 437 | 1.25 | .85 | 318 | 363 | 1.18 |
| 203 | Trebi | 416 | 631 | 1.30 | .66 | 394 | 493 | 1.28 |
| 204 | Peatland | 353 | 497 | 1.22 | .71 | 375 | 418 | 1.19 |
| 205 | Glabron | 292 | 382 | 1.26 | .76 | 263 | 325 | 1.18 |
| 206 | Wis. No. 38 | 228 | 315 | 1.25 | .72 | 228 | 283 | 1.11 |
| 207 | Odessa | 370 | 515 | 1.38 | .72 | 335 | 431 | 1.19 |
| 208 | Manchuria | 410 | 573 | 1.33 | .72 | 363 | 474 | 1.21 |
| | Average | 352 | 475 | 1.283 | .746 | 326 | 397 | 1.19 |

[1] Ratio B = $\frac{\text{Total amylase of green malt}}{\text{Total amylase of barley}}$.

[2] Ratio C = $\frac{\text{Free amylase of green malt}}{\text{Total amylase of green malt}}$.

[3] Ratio D = $\frac{\text{Total amylase of green malt}}{\text{Total amylase of dry malt}}$.

lase activity of malt on drying, presumably due to heat inactivation. The difference between free and total amylase activity decreased during germination and continued to decrease during drying of the malt.

The varieties that are high in diastatic activity for the season of 1935 are likewise high for the season of 1936. Trebi, Peatland, and Manchuria are consistently high in $\beta$-amylase activity and Wis. No. 38 is consistently low for both years.

## α-Amylase of Barley and Malt

Jozsa and Gore (1932), and Gore and Jozsa (1932) have demonstrated that normal wheat flour may contain a measurable quantity of α-amylase. Shellenberger and Bailey (1936) found variations in the

starch dextrinizing activity of 26 barley samples, although in most instances the activity was very low. These observations do not agree with Nordh and Ohlsson's (1932) conclusion that ungerminated barley contains no α-amylase activity.

An attempt was made to vary the activity of the two amylase functions of an extract of ungerminated barley using Ohlsson's (1926) technique for separating α- and β-amylases.

*Solution A:* 2.5 g. (dry basis) of Velvet barley were extracted with 50 c.c. of water and the extract diluted 1 : 10 with water.

*Solution B:* 25 c.c. of the original barley extract were acidified with 1.25 c.c. of 0.1 N HCl and kept at 0–5° C. for 15 minutes (pH 3.35). Then .75 c.c. of 0.2 M $Na_2HPO_4$ was added and 2.7 c.c. of this solution (pH 6.67) diluted to 25 c.c. with water.

*Solution C:* 20 c.c. of the orignial barley extract were heated to 60° C. for 15 minutes, cooled, and diluted 1 : 10 with water.

Acidification to pH 3.3 destroys most of the α-amylase activity with very little reduction in β-amylase, whereas heating to 60–70° C. impairs β-amylase more than α-amylase.

TABLE III

DETERMINATION OF α-AMYLASE IN UNGERMINATED BARLEY

| Enzyme solution | Mg. maltose per 50 c.c. | Wohlgemuth values |
|---|---|---|
| Sample No. 202 ..... Velvet 1936 Crop | | |
| 2 c.c. barley extract (Solution A) | 150 | .33 |
| 2 c.c. solution B | 122 | .33 |
| Sample No. 210 ..... Velvet 1935 Crop | | |
| 2 c.c. barley extract (Solution A) | 105 | 1.25 |
| 2 c.c. solution B | 95 | .13 |
| 2 c.c. solution C | 34 | 1.00 |

The sample of Velvet from the 1935 crop showed a separation of its α- and β-amylase functions by either acid (Solution B) or heat treatment (Solution C). The fact that it was possible to vary the two amylase functions independently of each other constitutes evidence that both α- and β-amylases are present in ungerminated barley.

The sample of ungerminated barley from the 1936 crop appears to contain no α-amylase activity. The acid treatment, which destroys α-amylase chiefly, caused no appreciable reduction in the Wohlgemuth value. The low starch-dextrinizing activity (0.33) of this sample was apparently due to the hydrolytic action of β-amylase. For this sample the starch-iodine color changed from blue to light blue to faint blue, or

colorless, whereas, for the sample No. 210, which contained a small amount of α-amylase, there was a distinct transition from blue to violet to red.

The α-amylase activity of ungerminated barley, green malt, and dry malt is given in Table IV.

TABLE IV

α-AMYLASE ACTIVITY OF BARLEY AND MALT. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

| | | C.c. of 2% soluble starch solution dextrinized per c.c. of barley extract on 1 hr. digestion, 40° C. | | | | |
|---|---|---|---|---|---|---|
| | | Barley meal | Green malt | | Dry malt | |
| Number | Variety | Free | Free | Total | Free | Total |
| | | 1935 Crop | | | | |
| 209 | Oderbrucker | 1.50 | 60 | 125 | 100 | 130 |
| 210 | Velvet | 1.25 | 85 | 125 | 86 | 107 |
| 211 | Trebi | .71 | 44 | 103 | 86 | 94 |
| 212 | Peatland | 3.08 | 65 | 120 | 111 | 115 |
| 213 | Glabron | 1.00 | 41 | 107 | 79 | 103 |
| 214 | Wis. No. 38 | .50 | 33 | 86 | 65 | 100 |
| 215 | Spartan | .50 | 28 | 63 | 54 | 65 |
| 216 | Minn. No. 462 | 1.20 | 36 | 73 | 70 | 88 |
| | Average | 1.22 | 49 | 100 | 81 | 100 |
| | | 1936 Crop | | | | |
| 201 | Oderbrucker | .42 | 104 | 143 | 125 | 125 |
| 202 | Velvet | .33 | 86 | 139 | 119 | 125 |
| 203 | Trebi | .41 | 81 | 154 | 114 | 119 |
| 204 | Peatland | .48 | 93 | 161 | 147 | 128 |
| 205 | Glabron | .44 | 81 | 104 | 100 | 139 |
| 206 | Wis. No. 38 | .38 | 61 | 106 | 91 | 122 |
| 207 | Odessa | .38 | 114 | 200 | 135 | 200 |
| 208 | Manchuria | .40 | 109 | 161 | 156 | 169 |
| | Average | .41 | 81 | 146 | 123 | 141 |

The barley samples from the 1935 crop contain a significant quantity of α-amylase, with the possible exception of the varieties Wis. No. 38 and Spartan. The green malt and dry malt from the 1935 crop are much lower in α-amylase activity than those from the following year. There was a slight increase in free α-amylase on drying of the malt but the total α-amylase activity changed very little, if any.

The barley samples grown in 1936 were very low in dextrinizing activity, the Wohlgemuth values ranging from .33 to .48. For each sample the starch-iodine color change was from blue to faint blue or colorless. Thus it is concluded that the samples of barley from the 1936 crop contain no α-amylase and that the loss of blue starch-iodine color was almost entirely due to the action of β-amylase.

The varieties, Odessa, Manchuria, Oderbrucker, and Peatland are high in α-amylase activity and Wis. No. 38 is low. There is a significant positive correlation between the total α- and total β-amylase activity of green malt ($r = 0.869 \pm 0.041$).

## Effect of α-Amylase Upon Saccharifying Activity of Green Malt

As was previously pointed out in this discussion, the increase in total β-amylase activity on germination of barley, as measured by maltose production, is approximately 25% to 29%. Klinkenberg (1932) observed that α-amylase acting on soluble starch hydrolyzed 36% of the starch to maltose. Therefore, it is conceivable that part of the increase in saccharifying activity on germination of barley may be due to the action of α-amylase appearing upon germination.

Freeman and Hopkins (1936) studied the rate of hydrolysis of soluble starch by α- and β-amylases acting separately and the combined effect of the two acting together. They found that in the earlier stages of hydrolysis, α-amylase produced almost as much maltose as did an equivalent amount of β-amylase. A mixture of α- and β-amylase in low concentration functioned additively in hydrolyzing starch but when the ratio of α- to β-amylase was low the combined rate was greater than the sum of the two component rates.

These results would suggest that the increase in total β-amylase activity (maltose production) on germination is partly due to the effect of α-amylase and that the β-amylase concentration may increase very little, if any. In order to test this explanation, a solution of purified α-amylase was added to a water extract of Velvet (1936) barley, which was observed to be free of β-amylase, and the increase in maltose production noted.

The purified α-amylase preparation used was prepared by Olof Stamberg, of the Division of Agricultural Biochemistry, University of Minnesota. A solution was prepared by triturating 50 mg. of dry preparation with a few cubic centimeters of water in a mortar and diluting to 50 c.c. volume. This solution was further diluted 1 : 5 with water so that the final concentration was 0.20 mg. per cubic centimeter. The ratios of β- to α-amylase employed are in the range of the observed ratio of the two enzymes in the green malt samples.

The ratios of β- to α-amylase of the three solutions examined are given in the last column of Table V. The solutions of purified α-amylase produced an appreciable quantity of maltose. The amount of maltose produced by α- and β-amylases acting together was approximately the sum of the two amounts produced by their acting separately.

By using the data in Table V together with the values for the average

TABLE V

EFFECT OF α-AMYLASE UPON MALTOSE PRODUCTION BY β-AMYLASE

| Enzyme addition | C.c. 2% starch dextrinized by α-amylase | Mg. maltose | Increase due to α-amylase: Mg. maltose | Increase due to α-amylase: % | Ratio β/α |
|---|---|---|---|---|---|
| 2.1 c.c. α-amylase solution | 6.0 | 17 | | | |
| 2.8 c.c. α-amylase solution | 8.0 | 21 | | | |
| 4.2 c.c. α-amylase solution | 12.0 | 29 | | | |
| 1.6 c.c. of β-amylase solution | | 124 | | | |
| 1.6 c.c. β + 2.1 c.c. α | 6.0 | 142 | 18 | 14.52 | 20.67 : 1 |
| 1.6 c.c. β + 2.8 c.c. α | 8.0 | 151 | 27 | 21.77 | 15.50 : 1 |
| 1.6 c.c. β + 4.2 c.c. α | 12.0 | 162 | 38 | 30.64 | 10.33 : 1 |

α- and β-amylase activities of the samples from the two crops, it was possible to calculate the increase in maltose production due to the increase in α-amylase activity on germination. These calculations are shown in Table VI. This calculated increase was compared with the observed increase in saccharifying activity.

TABLE VI

CALCULATED INCREASE IN SACCHARIFYING ACTIVITY DUE TO α-AMYLASE APPEARING ON GERMINATION. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

| Number | | Average 1935 crop | Average 1936 crop |
|---|---|---|---|
| 1 | Total β-amylase activity of barley (per .2 c.c.) | 237 | 369 |
| 2 | Total β-amylase activity of green malt (per .2 c.c.) | 296 | 475 |
| 3 | Apparent increase on germination. No. 2/No. 1 | 24.89% | 28.73% |
| 4 | Total α-amylase activity of green malt (per 1.0 c.c.) | 100 | 145 |
| 5 | Total α-amylase activity per .2 c.c. | 20 | 29 |
| 6 | Ratio $\frac{\text{Total } \beta \text{ of barley}}{\text{Total } \alpha \text{ of malt}}$ | 11.85 : 1 | 12.72 : 1 |
| 7 | Calculated increase in maltose production by addition of α-amylase to β-amylase in the ratio found in germinated barley (No. 6) By interpolating from values in Table V..... | 27.53% | 26.50% |

The calculated increase in maltose production shown in Table VI was obtained by interpolating between the values given in Table V for the relationship between the ratio of β- and α-amylases and the per cent increase in maltose production. This calculated increase in β-amylase activity (maltose production) agrees closely with the actual observed increase. These results lead to the conclusion that the concentration of total β-amylase of barley does not change on germination and that the

apparent increase in β-amylase activity, as measured by the rate of maltose production, is due, in reality, to the increase in α-amylase activity.

## Summary

The barley samples grown in 1936, an abnormally dry year, were much higher in protein content and lower in 1,000-kernel weight than the same varieties grown in 1935, a season having better growing conditions. The diastatic activity of the barley and malt from the 1936 crop was approximately 50% higher than that from the previous year's crop.

Contrary to the opinion of various investigators, sound ungerminated barley may contain a measurable amount of α-amylase. The barley samples from the 1936 crop were lacking in α-amylase but most of the samples grown in 1935 contained appreciable quantities of α-amylase.

A high positive correlation was observed between the total β-amylase activity of barley and of green malt, which suggests the possibility of using the β-amylase activity of a papain digest of barley to predict the β-amylase activity of the malted grain. There was also a significant positive correlation between total α-amylase and total β-amylase of green malt.

A comparison of the barley varieties in regard to both diastatic functions showed that Odessa, Manchuria, and Oderbrucker were high, and Wis. No. 38, Spartan, Minn. No. 462, and Glabron were low. Trebi was highest in β-amylase but was deficient in α-amylase activity.

Germination of barley resulted in a relatively large increase in free (water-soluble) β-amylase activity but the increase in total β-amylase activity was only 25% to 29%. However, it was found that the concentration of total β-amylase did not change on germination of barley and that the observed increase in total β-amylase activity, as measured by the rate of maltose production, was due to the increase in α-amylase activity.

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# SOME OBSERVATIONS ON MALTOSE PRODUCTION IN FLOUR AND DOUGH[1]

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It is well known that more than one kind of sugar is normally present in flour and that sugar production in dough continues during fermentation.

Cane sugar is present in all wheat flours sometimes to the extent of 2 to 3%. Reducing sugars are present in very small quantities only. During panary fermentation sugars of the maltose type must be produced because such sugars only are directly acted upon by yeast with the production of carbon dioxide. Without such sugars, or with an inadequate supply, insufficient gas would be produced by the yeast, especially in the critical or proving period of fermentation, and only small, heavy, stodgy loaves would result.

Production of sugars during fermentation takes place in two ways: (1) from the starch by the action of *diastase* present in the *flour*, and (2) from the cane sugar present through the action of *invertase* present in the *yeast*. Thus, flour diastase converts some starch into maltose which is then converted into glucose by the maltase present in yeast. On the other hand, invertase present in yeast will convert cane sugar into glucose and fructose which may then be converted into alcohol and carbon dioxide by the zymase of the yeast.

As is well known, flours vary widely in gas-producing capacity. Some produce a steady and large supply of gas throughout the whole fermentation period. Others will produce a steady but smaller supply. Others again may gas freely in the earlier stages of fermentation but gas production may slow up later, so that in the critical or proving period of fermentation insufficient gas may be produced to properly inflate the dough, small, stodgy loaves resulting.

These differences in rate of gas production are due to several factors, one of which is the variation that exists in the *diastatic capacity* of different flours. The greater the diastatic capacity the greater will be the rate of production of maltose from starch, and the greater, therefore, will be the production of carbon dioxide from maltose by

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the yeast. If the diastatic capacity of a flour is too low, gas production may still be rapid in the earlier stages of fermentation because this early gas is produced from the sugars already existing in the flour. When this pre-formed sugar in such low diastatic flours has been used up, the rate of gas production will fall rapidly and may become entirely inadequate in final proof. In such cases gas production during final proof may be maintained at a high and satisfactory rate by increasing the diastatic power of the flour by the addition to the flour of small quantities (*e.g.*, 0.1% = 4½ ounces per sack of 280 pounds) of highly diastatic malt extract. Gas production can be increased still further in some cases by adding with the malt extract a further 0.05% (or 2¼ ounces per sack) of ammonium phosphate. This is illustrated in

TABLE I

EFFECT OF MALT EXTRACT AND AMMONIUM PHOSPHATE ON RATE OF GAS PRODUCTION OF VARIOUS FLOURS

50 g. flour, 1 g. (= 2%) yeast, 0.625 g. (= 1¼%) salt, 30 c.c. water, and temperature of fermentation of 80° F.

| | No. 2 N. Manitoba | | Garnet | | No. 1 Hard Winter | |
|---|---|---|---|---|---|---|
| | | M.E.[1] +A.P. | | M.E.[1] +A.P. | | M.E.[1] +A.P. |
| | *C.c.* | *C.c.* | *C.c.* | *C.c.* | *C.c.* | *C.c.* |
| 1st hour | 31 | 41 | 30 | 47 | 43 | 30 |
| 2nd " | 78 | 84 | 72 | 79 | 74 | 67 |
| 3rd " | 89 | 102 | 83 | 102 | 83 | 83 |
| 4th " | 106 | 121 | 106 | 122 | 97 | 101 |
| 5th " | 108 | 127 | 71 | 137 | 113 | 116 |
| 6th " | 82 | 117 | 34 | 93 | 106 | 120 |
| 7th " | 51 | 90 | 24 | 50 | 55 | 103 |
| 8th " | 36 | 53 | 16 | 37 | 35 | 56 |
| Total gas in 24 hours | 840 | 1099 | 516 | 983 | 870 | 1000 |

[1] 0.1% malt extract and 0.05% ammonium phosphate.

Table I and Plate I. It will be noticed from Table I that, with 2% yeast and at 80° F., the No. 1 Hard Winter flour gassed freely and at an increasing rate for 6 hours, the No. 2 N. Manitoba for 5 hours, and the Garnet flour for only 4 hours. With malt extract and ammonium phosphate present rate of gas production was not materially increased in the first two hours, but was increased in the third and subsequent hours, and the high rate was maintained for 6 or 7 hours. This effect was most marked with Garnet flour, the poorest "gasser" of the three, and least with Hard Winter, the best gasser.

The Manitoba and Garnet flours were baked using the same formula of 2% yeast, 1¼% salt, fermentation temperature 80° F., and

absorption 16 gallons of liquor per sack. Four sets of doughs, each consisting of three doughs made at $\frac{3}{4}$-hour intervals, were made: two sets from the Manitoba (one set without and one set with malt extract

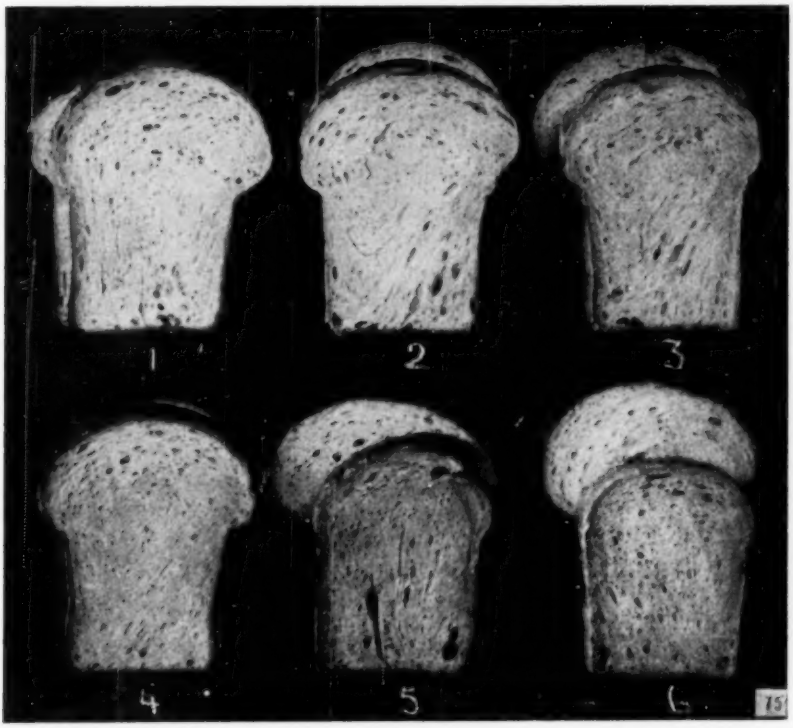


PLATE 1

The front loaves contain no malt extract and ammonium phosphate. The rear loaves contain 0.1% (of weight of flour) of malt extract and 0.05% of ammonium phosphate.

1 front = A1 = No. 2 Manitoba, without malt extract and ammonium phosphate, 4 hours to oven.
2 " A2 " " " " " " " " " " " " " "
3 " A3 " " " " " " " " " " $5\frac{1}{4}$ " " "

1 rear = B1 = No. 2 Manitoba, with M.E. and A.P., 4 hours to oven.
2 " B2 " " " " " " $4\frac{1}{4}$ " " "
3 " B3 " " " " " " $5\frac{1}{4}$ " " "

4 front = C1 = Garnet, without M.E. and A.P., 4 hours to oven.
5 " C2 " " " " " " $4\frac{1}{4}$ " " "
6 " C3 " " " " " " $5\frac{1}{4}$ " " "

4 rear = D1 = Garnet, with M.E. and A.P., 4 hours to oven.
5 " D2 " " " " " " $4\frac{1}{4}$ " " "
6 " D3 " " " " " " $5\frac{1}{4}$ " " "

and ammonium phosphate) and two sets from the Garnet flour (one set without and one set with malt extract and ammonium phosphate). Thus the following loaves were baked together:

A1. Manitoba, without malt extract, 4 hours to oven.
A2. " " " " 4¾ " " "
A3. " " " " 5½ " " "
B1. " with malt extract and ammonium phosphate, 4 hours to oven.
B2. " " " " " " " 4¾ " " "
B3. " " " " " " " 5½ " " "
C1. Garnet, without malt extract and ammonium phosphate, 4 " " "
C2. " " " " " " " 4¾ " " "
C3. " " " " " " " 5½ " " "
D1. " with malt extract and ammonium phosphate, 4 hours to oven.
D2. " " " " " " " 4¾ " " "
D3. " " " " " " " 5½ " " "

It will be seen from Plate 1 how closely similar in volume are A1 and B1, and C1 and D1; that is, where gas production is already adequate malt extract may have little effect on loaf volume. When gas production is deficient malt extract, especially with the addition of ammonium phosphate, may produce a large effect on loaf volume, the effect being the greater the greater the gas deficiency. The loaves of the C series decreased greatly in volume from C1 to C3 whereas the D series actually increased in volume from D1 to D3. The Garnet flour benefited more than the Manitoba through the addition of malt extract.

It is obvious that no conclusions whatever concerning the relative baking qualities of Garnet and Manitoba, or for that matter of any series of flours, can be drawn unless and until adequate gas production is assured in each case. In all experimental work a naturally poor or unsatisfactory "gasser" must be corrected, as far as possible, by treatment with malt extract and ammonium phosphate.[2] In commercial practice inadequate gas production in some flours (such as certain Plate, English and some American wheats) is corrected by blending with exceptionally good gassers, such as durum or low grade Manitobas.

The importance of gas production as a separate factor in the quality problem arose out of the work of Wood (1907). Methods of controlling it by the addition of malt extract and ammonium phosphate and in other ways were subsequently worked out by Humphries who has emphasized the supreme practical importance of the point in the numerous Reports of the Home Grown Wheat Committee of the National Association of British and Irish Millers from 1907 onwards.

Two very extensive investigations on the diastatic capacity of flours and its relation to the "strength" problem were carried out by Rumsey (1922) and by Collatz (1922). These workers added considerably to our knowledge of the problem and Rumsey developed a method of estimating the rate of formation of maltose in a flour. For further work on this problem see also Jørgensen (1931), Blish, Sand-

[2] Two per cent. of cane sugar will have a similar effect.

stedt, and Astleford (1932), Steller, Markley, and Bailey (1935) and Landis and Frey (1936). A modification of this method introduced by Kent-Jones (1924), although less exact than the original method, is simple in technique, can be carried out by a comparatively inexperienced worker, and has proved itself to be of considerable value in the routine examination of flour.

The method consists of mixing 30 g. of flour with 160 c.c. of water, keeping the mixture at 27° C. for one hour, and then determining the amount of reducing sugar present in the mixture. The figure obtained is called the *maltose figure* and is a measure of the amount of maltose produced, in the particular conditions of the experiment, in one hour together with the small but variable amount of maltose initially present in the flour. The latter is usually so small that it can be ignored without impairing the practical value of the determination.

From what has been written above concerning the importance of maltose production as a factor in gas production in doughs, it might at first appear that the maltose figure should afford a measure of gas production also, in which case the long, tedious and cumbersome gassing test could be replaced by the short and simple maltose test. Kent-Jones (1924) indeed is of this opinion for he states:

> "There is a definite connection between the maltose figure and gas production. Flours which give high maltose figures give great gas production, and moreover, still continue to give large quantities of gas after some hours. Those flours which have . . . low maltose figures may give in the initial stages a sufficiency of gas, but fail to do so later on at the critical period."

Kent-Jones recommends that the maltose test should be used as a substitute for the gas test, but the very few data available in the literature are altogether insufficient to establish the relationship claimed. There are other factors, besides maltose production, that contribute to adequate gas production in doughs, and in point of fact it has been found in the writers' laboratories that many flours of relatively low maltose figure behave satisfactorily in the bakehouse as regards gas production.

In an attempt to ascertain the precise relation between maltose figure and gas production, a large number of determinations of both have been made on a variety of flours and the results examined statistically. Correlation coefficients have been calculated connecting maltose figures with gas production (*a*) in 24 hours, (*b*) in the whole fermentation period, and (*c*) during final proof, with two different proportions of yeast. The results are given in Table II. No significant correlation was found between maltose figure and proving gas nor between maltose figure and total gas produced during the entire fermentation period (including final proof). This lack of correlation

occurs with long and with short fermentations, *e.g.*, 8 hours with $\frac{1}{2}\%$ yeast and $3\frac{1}{4}$ hours with 2% yeast. The only definite correlation found was between maltose figure and total gas produced in 24 hours—a correlation that is of little practical importance.[3]

TABLE II

CORRELATION COEFFICIENTS BETWEEN MALTOSE FIGURES AND GAS PRODUCTION

| | Correlation coefficient | Probable error (P.E.) of the coefficient | P.E. × 6 | Number of tests |
|---|---|---|---|---|
| Total gas production in 24 hours using $\frac{1}{2}\%$ yeast | 0.695 | ±0.040 | .240 | 75 |
| Total gas production in 8 hours using $\frac{1}{2}\%$ yeast | 0.060 | ±0.078 | .468 | 71 |
| Total gas production in $3\frac{1}{4}$ hours using 2% yeast | −0.273 | ±0.072 | .432 | 73 |
| Gas production in 8th hour, using $\frac{1}{2}\%$ yeast | −0.023 | ±0.082 | .492 | 66 |
| Gas production between $2\frac{1}{2}$ and $3\frac{1}{4}$ hours, using 2% yeast | 0.281 | ±0.070 | .420 | 75 |

This lack of correlation is surprising in view of the known importance of maltose production in panary fermentation and especially when one remembers the ease with which gas production can be stimulated by the addition of malt extract to flour, *i.e.*, by increasing diastatic activity. The fact that the maltose test is carried out under conditions widely different from those that obtain in a dough may have a bearing on the problem. There is no *a priori* reason why maltose production should take place in a mixture of 30 g. of flour and 15 g. of water plus yeast and salt (*i.e.*, in a dough) at the same rate as in a mixture containing ten times as much water, and in the absence of yeast and salt. Yet this tacit assumption lies at the basis of the maltose test. Rumsey found that the maltose figure, as determined by his method, was independent of the amount of water used provided the water was at least equal in amount to the flour. If less than this minimum amount of water were present, as in a dough, the maltose figure might be reduced by as much as 20% (see Table III, section A). The Kent-Jones method of estimation is only approximate and its accuracy does not extend beyond the first decimal place. Determinations carried out by this method (see Table III, section B) in these laboratories were not conclusive, but do suggest a diminished

[3] The high correlations found by Larmour, Geddes, and Whiteside (1933), Davis and Worley (1934) and by Graesser (1936) were between diastatic activity and *total* gas production using a *very* large excess of yeast. Such gas figures bear little relation to those obtained in fermenting doughs under ordinary commercial conditions. The object of the present investigation was to study the relationship of the maltose figure, as conventionally determined in England, to rate of gas production in ordinary panary fermentation with a view to seeing whether a test of diastatic activity can be used as a substitute for the cumbrous gassing test.

rate of maltose formation in dough as compared with more dilute flour-water mixtures. The effect, however, is small and is probably not a main factor in determining the absence of correlation between maltose figure and gas production.

TABLE III

EFFECT ON MALTOSE FIGURE OF VARYING THE WATER CONTENT OF THE FLOUR-WATER MIXTURE

A.—L. A. Rumsey—Using Rumsey's Method for Estimation of Maltose:—

| Flour-water ratio | Maltose figure (%) |
|---|---|
| 1 : 0.55 | 2.171 |
| 0.7 | 2.358 |
| 1.0 | 2.790 |
| 2.5 | 2.722 |
| 7.5 | 2.716 |
| 10.0 | 2.714 |
| 40.0 | 2.716 |

B.—E. A. Fisher—Using Kent-Jones' Approximate Method for Estimation of Maltose:—

| Flour | Water | Flour-water ratio | Maltose figure | |
|---|---|---|---|---|
| | *C.c.* | | *After 1 hour* | *After 7 hours* |
| 30 g. | 15 | 1 : 0.5 | 1.13 | 1.76 |
| | 30 | 1.0 | 1.41 | 2.46 |
| | 60 | 2.0 | 1.21 | 2.29 |
| | 85 | 2.83 | 1.19 | 2.31 |
| | 110 | 3.66 | 1.31 | 2.07 |
| | 130 | 4.33 | 1.18 | 2.36 |
| | 160 | 5.33 | 1.17 | 1.98 |

It has been established by several investigators (Rumsey, 1922; Collatz, 1922), and confirmed in the writers' laboratories, that in the absence of yeast reducing sugars will steadily accumulate in a dough. With yeast present maltose figure will increase for the first hour or so and will indeed usually exceed that obtained in the absence of yeast (owing to the production of glucose and fructose from cane sugar by yeast). After an hour or so, when the yeast has become fully active, maltose figure will decrease steadily throughout fermentation owing to the fact that the reducing sugars are utilized by the yeast faster than they are produced. The figures quoted in Table IV sufficiently establish this point.

It seemed possible that some light might be thrown on the problem by determining maltose figures at hourly intervals in the presence as well as in the absence of yeast. The results for two flours, *A* and *B*, both straight-run commercial flours, and for two different brands of yeast, are given in Table V. It is surprising that the yeast, instead of

TABLE IV

AMOUNTS OF REDUCING SUGARS (MALTOSE) CALCULATED AS PERCENTAGES OF DOUGH PRESENT IN DOUGH AT DIFFERENT STAGES OF FERMENTATION

| Time | Percentage maltose | | Time | Percentage maltose | |
|---|---|---|---|---|---|
| | No yeast | 2½% yeast | | No yeast | Approximately 3% yeast |
| | Rumsey | | | Collatz | |
| At mixing | 1.257 | 1.560 | At mixing | 0.67 | 0.90 |
| 1 hour | 1.594 | 2.095 | 1 hour | 1.085 | 1.405 |
| 2 hr. 57 min. | 2.041 | 2.055 | 2 hr. 30 min. | 1.390 | 1.500 |
| 4 " 12 " | 2.220 | 1.483 | 3 " 40 " | 1.54 | 1.380 |
| 4 " 57 " | 2.336 | 1.252 | 4 " 35 " | 1.76 | 1.095 |
| 5 " 59 " | 2.453 | .846 | 5 " 8 " | 1.745 | 1.015 |

| Time | Percentage maltose |
|---|---|
| | 2% yeast Fisher |
| At mixing | — |
| 1 hour | 1.545 |
| 2 hours | 1.070 |
| 3 " | .300 |
| 4 " | Nil |
| 5 " | " |

reducing the maltose figure, as it does in a dough, actually increased it, the increase being progressive throughout the whole period of incubation. That is, under the conditions of the maltose test (large excess of water being present) yeast *in*creases rate of production of reducing sugars, while in a dough yeast destroys these sugars as fast as or faster than they are produced.

To explore the problem further, gas-production figures and maltose figures were obtained at hourly intervals on a series of flour-water-yeast mixtures of varying water content. In Table VI are given gas-production figures for 30 g. of flour, 0.33 g. of yeast and varying amounts of water up to 160 c.c., the fermentation temperature being maintained at 26.7° C. In Figures 1 and 2 are given the hourly maltose figures for a similar series of mixtures only this time at the slightly different temperature of 27° C.

It will be noticed from Table VI that the rate of gas production is slightly greater when the flour and water concentrations are in the ratio 1 : 1 than when the concentrations are those in a dough, *viz.*,

2 : 1. When, however, the ratio of water to flour is further increased, the rate of gas production falls off progressively.

TABLE V

MALTOSE DETERMINATIONS AT HOURLY INTERVALS, WITH AND WITHOUT YEAST

*Flour A*

| Time | Maltose figure | |
|---|---|---|
| | Without yeast | + 1% D.C.L. yeast |
| 1 hour | 1.17 | 1.25 |
| 2 hours | 1.33 | 1.47 |
| 3 " | 1.48 | 1.69 |
| 4 " | 1.66 | 1.94 |
| 5 " | 1.80 | 2.24 |
| 5½ " | 1.92 | — |
| 5⅔ " | — | 2.53 |
| 6¼ " | 1.90 | 2.57 |
| 7 " | 1.98 | 2.81 |

*Flour B*

| Time | Maltose figure | | |
|---|---|---|---|
| | Without yeast | + 1% D.C.L. yeast | + 1% N.G. & S.F. yeast |
| 1 hour | 1.70 | 1.74 | 1.94 |
| 2 hours | 1.83 | 1.93 | 2.39 |
| 3 " | 2.00 | 2.16 | 2.81 |
| 4 " | 2.24 | 2.41 | 3.17 |
| 5 " | 2.40 | 2.69 | 3.50 |
| 5½ " | 2.47 | — | 3.63 |
| 6 " | 2.55 | 2.97 | 3.90 |
| 7 " | 2.59 | 3.28 | 4.16 |

An examination of Figures 1 and 2 shows that the rate of maltose production changes in a characteristic manner with changing water content. With 70 c.c. of water per 30 g. of flour, the maltose content

TABLE VI

GAS PRODUCTION FROM 30 G. FLOUR + VARYING AMOUNTS OF WATER AND 0.3 G. (= 1%) YEAST

| Water in dough..... | 15 c.c. | 30 | 60 | 70 | 85 | 110 | 130 | 160 |
|---|---|---|---|---|---|---|---|---|
| | *C.c.* | | | Gas figures | | | | |
| 1 hour..... | 11 | 6 | 4 | 1 | 1 | 0 | 1 | 2 |
| 2 hours..... | 30 | 19 | 10 | 6 | 4 | 4 | 3 | 4 |
| 3 " ..... | 56 | 50 | 29 | 25 | 17 | 12 | 11 | 11 |
| 4 " ..... | 92 | 95 | 66 | 59 | 55 | 38 | 31 | 38 |
| 5 " ..... | 140 | 150 | 120 | 105 | 83 | 65 | 51 | 55 |
| 6 " ..... | 192 | 210 | 168 | 151 | 127 | 89 | 67 | 65 |
| 7 " ..... | 238 | 253 | 198 | 181 | 164 | 109 | 85 | 73 |
| 8 " ..... | 264 | 276 | 220 | 205 | 176 | 123 | 97 | 79 |
| 24 " ..... | 438 | 492 | 452 | 444 | 412 | 233 | 139 | 95 |

of the suspension remained substantially the same from one to seven hours. With more water than this the maltose figure increased with increasing time, the rate of increase being greater the greater the content of water. On the other hand, with less water than 70 c.c. the maltose figure, although increasing during the first few hours, fell off rapidly with further increases in time. This fall-off was greater the lower the water content of the mixture.

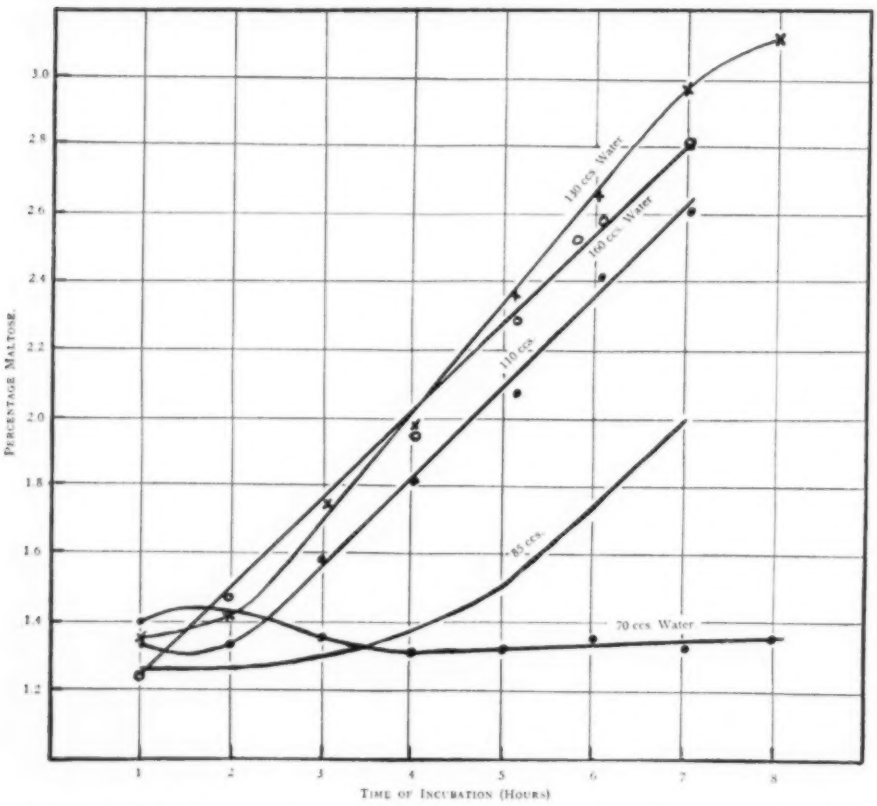


Figure 1. Hourly Maltose Production from 30 Grs. of Flour A + 1% D.C.L. Yeast and Varying Amounts of Water.

The reducing sugar content of a suspension at any given time is dependent on the balance between the sugar produced by the inversion of the original sucrose present and by the hydrolysis of the starch on the one hand, and the sugar used up by the yeast on the other hand. It is thus significant that the accumulation of maltose is greater in dilute suspensions where gas production is less, and is smaller where the flour concentration and therefore also gas production are greater.

To obtain an idea of the actual reducing sugar (expressed as mal-

tose) produced in these flour-water-yeast mixtures, the maltose consumed in the production of gas was calculated from the equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

and on this basis Table VII was constructed. It is evident that the

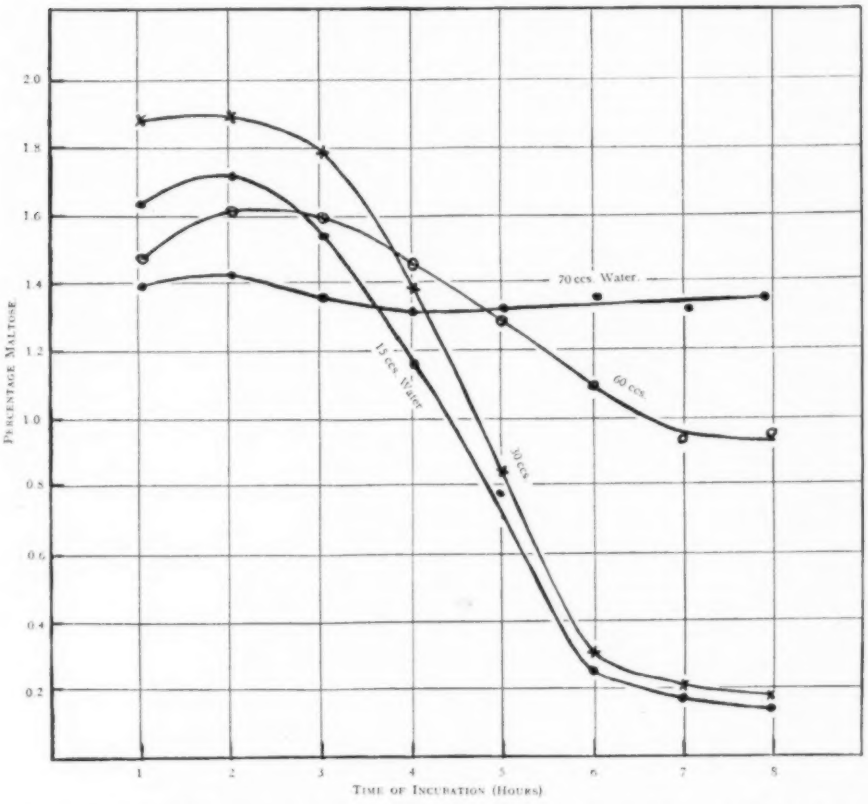


Figure 2. Hourly Maltose Production from 30 Grs. of Flour A + 1% D.C.L. Yeast and Varying Amounts of Water.

actual amount of maltose produced in any given suspension after any given time is equal to the sum of the maltose consumed in gas production and that remaining unused.

From some of the data in Table VII, Figure 3 was drawn. The curves in this figure corresponding to water contents of 15 and 30 c.c. per 30 g. of flour are apparently linear, and since with such low water concentration the inversion of the initial sucrose is almost complete within the first hour, it is probable that the slope of these curves gives a measure of the rate of maltose production through enzymic hydrolysis of starch. With more dilute mixtures the inversion of the sucrose

TABLE VII

MALTOSE FIGURES (REAL AND THEORETICAL) AT HOURLY INTERVALS OF FLOUR *A* + 1% D.C.L. YEAST

| C.c. $H_2O$ per 30 g. flour | 15 | | | 30 | | | 60 | | | 70 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Hours* | *A*[1] | *B*[2] | *C*[3] | *A* | *B* | *C* | *A* | *B* | *C* | *A* | *B* | *C* |
| 1 | .13 | 1.63 | 1.76 | .07 | 1.87 | 1.94 | .05 | 1.47 | 1.52 | .01 | 1.39 | 1.40 |
| 2 | .35 | 1.71 | 2.06 | .22 | 1.89 | 2.11 | .12 | 1.61 | 1.73 | .07 | 1.42 | 1.49 |
| 3 | .66 | 1.53 | 2.19 | .59 | 1.78 | 2.37 | .34 | 1.59 | 1.93 | .29 | 1.35 | 1.64 |
| 4 | 1.08 | 1.18 | 2.26 | 1.12 | 1.34 | 2.46 | .78 | 1.45 | 2.23 | .70 | 1.31 | 2.01 |
| 5 | 1.65 | .81 | 2.46 | 1.77 | .95 | 2.72 | 1.42 | 1.32 | 2.74 | 1.24 | 1.32 | 2.56 |
| 6 | 2.27 | .25 | 2.52 | 2.48 | .29 | 2.77 | 1.98 | 1.09 | 3.07 | 1.78 | 1.34 | 3.12 |
| 7 | 2.81 | .17 | 2.98 | 2.98 | .20 | 3.18 | 2.34 | .95 | 3.29 | 2.14 | 1.33 | 3.47 |
| 8 | 3.12 | .14 | 3.26 | 3.26 | .18 | 3.44 | 2.60 | .94 | 3.54 | 2.42 | 1.35 | 3.77 |
| 24 | 5.17 | | | 5.20 | | | 5.32 | | | 5.23 | | |
| | 85 | | | 110 | | | 130 | | | 160 | | |
| 1 | .01 | 1.26 | 1.27 | 0 | 1.33 | 1.33 | .01 | 1.35 | 1.36 | .02 | 1.25 | 1.27 |
| 2 | .05 | 1.28 | 1.33 | .05 | 1.33 | 1.38 | .04 | 1.41 | 1.45 | .05 | 1.47 | 1.52 |
| 3 | .20 | 1.27 | 1.47 | .14 | 1.53 | 1.67 | .13 | 1.72 | 1.85 | .13 | 1.69 | 1.82 |
| 4 | .65 | 1.34 | 1.99 | .45 | 1.76 | 2.21 | .37 | 1.96 | 2.33 | .45 | 1.94 | 2.39 |
| 5 | .98 | 1.51 | 2.49 | .76 | 2.00 | 2.76 | .60 | 2.31 | 2.91 | .65 | 2.24 | 2.89 |
| 6 | 1.50 | 1.72 | 3.22 | 1.02 | 2.39 | 3.41 | .79 | 2.62 | 3.41 | .77 | 2.53 | 3.30 |
| 7 | 1.93 | 1.99 | 3.92 | 1.29 | 2.37 | 3.66 | 1.01 | 2.96 | 3.97 | .86 | 2.57 | 3.43 |
| 8 | 2.08 | — | — | 1.45 | — | — | 1.14 | 3.12 | 4.26 | .93 | 2.81 | 3.74 |
| 24 | 4.86 | | | 2.76 | | | 1.64 | | | 1.06 | | |

[1] *A* = maltose consumed by yeast (calculated).
[2] *B* = maltose remaining in solution.
[3] *C* = total maltose produced (= *A* + *B*).

is more gradual (see Table V) and in consequence the maltose content of such mixtures is lower during the first few hours than in the case of the more concentrated mixtures.

It was shown in Table III that the production of maltose from starch in a flour-water mixture is almost independent of the flour concentration. If this were equally true for flour-water-yeast mixtures the curves in Figure 3 should, after sufficient time had been allowed for the complete inversion of the sucrose in each case, all reach the same maltose level. Since this is not the case, and the curves for the lower flour concentrations actually cross those for the higher flour concentrations reaching a much higher maltose level after 6 to 7 hours, it is apparent that the rate of production of maltose in flour-water-yeast mixtures is not independent of water content but increases with it.

The observations recorded in this paper are of a purely preliminary character. They have not solved the problem of the lack of correla-

tion between maltose figure and gas production during fermentation, but have exposed to view a number of other problems, important in their categories, for future investigation. The observations lend support to the view that the problems of panary fermentation are vastly more complex than may appear at first sight, and that the lack of correlation between maltose figure and gas-producing capacity is due to the wide differences in the conditions under which the maltose test is carried out and those obtaining in a dough.

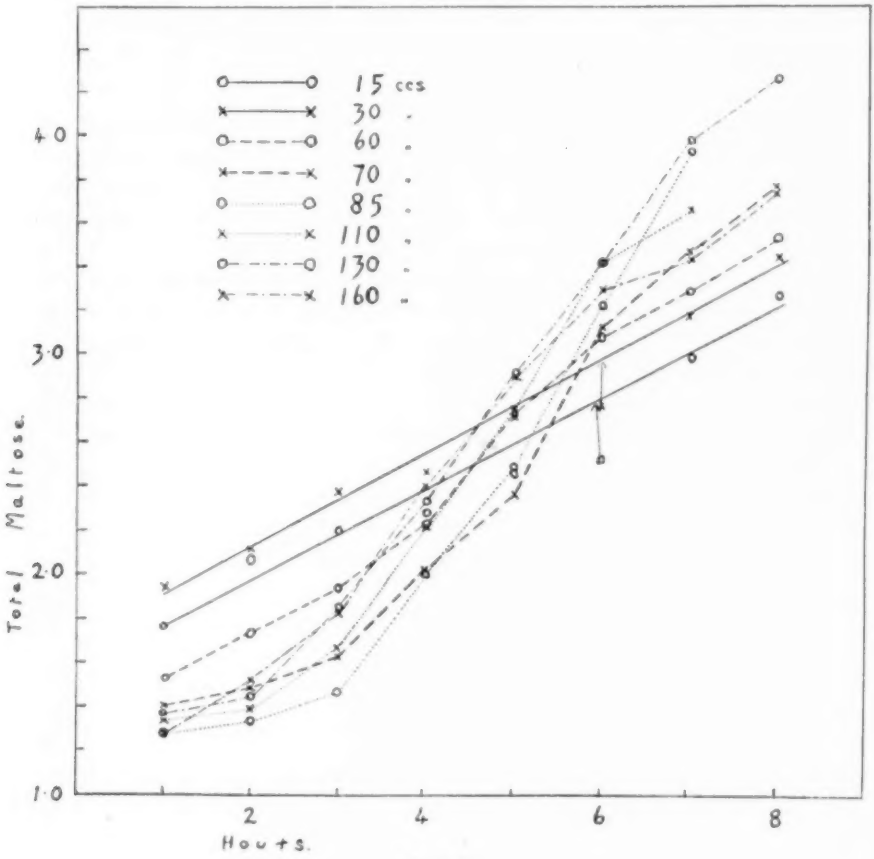


Figure 3.

It follows from this work that the maltose test cannot be used as a safe substitute for the gas test, and that a low maltose figure is not necessarily undesirable. The real importance of the maltose test lies at the other end of the scale: as shown in the writers' laboratories in 1926, it is flours of high maltose content, rather than low maltose flours, that are likely to give trouble in the bakehouse. Flours of

maltose figures higher than about 2.3 produce sticky doughs, the loaves refusing to bake or "soak."

## Summary

The importance of diastatic activity of flour in its relation to adequate gas production during fermentation is emphasized. Diastatic action results in the formation in dough of maltose which is acted on by yeast with the production of alcohol and carbon dioxide. On this account some workers consider that rate of maltose production should afford a measure of the rate of gas production and have suggested that the familiar maltose test can be used as a substitute for the tedious and cumbersome gassing test. It is shown that there is no correlation at all between the maltose figure, as conventionally determined, and gas production either during final proof or throughout the whole course of fermentation, in either short or long doughs. This complete absence of correlation between maltose figure and gas production is surprising and attempts have been made to explain it. The experimental work described has not completely solved the problem, but has indicated that the absence of correlation is due probably to the wide differences that exist between the conditions under which the maltose test is carried out and those obtaining in a dough. In the course of the work a number of fresh problems, important in their respective categories, have been exposed for future investigation.

As a result of this work it is emphasized that the routine maltose test cannot be used as a safe substitute for the gas test. The real value of the maltose test is due to the dangers inherent in flours of high maltose content.

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# THE ISOLATION OF THE COMPOUND GIVING YELLOW CORN ITS CHARACTERISTIC ODOR[1]

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In connection with another problem, it has been observed that saponification of fresh yellow corn oil does not destroy its characteristic odor. The non-saponifiable matter contains, among other things, sterols, the pigments, and the odoriferous substances. The study of these latter compounds has been initiated with the aim of first finding a convenient method of isolation from whole corn, second, localizing them in the corn kernel, and third, determining their chemical structures.

The substances seem to be present in the oil and to give to it the characteristic odor whether it is obtained by pressing or extracting. Likewise, corn from which all the oil has been extracted no longer possesses the odor. This would lead one to conclude that the odoriferous substances may be a part of the oil. Furthermore, the parts of the corn which contain little or no oil possess little or no odor. Although the odor can be removed from the oil by steam distillation and appears apparently unchanged in the condensate, it cannot be obtained from whole corn by this method.

The odoriferous material can be obtained by direct steam distillation of the oil or of the non-saponifiable part of the oil. The former appears to be the best, since the saponification of large amounts of oil and especially the working up of the resulting soaps is an arduous task. Accordingly, a trial run was made using 375 grams of oil that had been extracted from ground yellow corn by means of ethyl ether. This oil

[1] Journal Paper No. J-530 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 567.

was steam-distilled until no appreciable odor was left. The distillate, 1500 c.c., possessed a pungent odor very suggestive of yellow corn. It also contained some free fatty acids and so was made alkaline with sodium hydroxide and steam-distilled again. During this distillation the odorous substances passed over much more rapidly than from the original oil. By repeated partial distillation of each condensate, the material was concentrated into a final condensate of 40 c.c. From this solution the odorous substance was removed with ether. It was then dried over solid KOH, decanted, and evaporated off. The residue was a very small quantity of a light yellow oil. This oil was distilled up to 220° C., yielding 6 milligrams of a nearly colorless liquid and leaving a brown waxy residue. Although the distillate was definitely a mixture, it was analyzed and found to contain 76.77% C and 11.65% H. It contained no N nor S. Its molecular weight, using bornyl bromide as the cryoscopic medium, was found to be 260. The substance possessed a powerful odor, the slightest trace of it on a glass thread being noticeable throughout the room.

A larger run was made using 10 liters of pressed oil. After the above process of concentration and extraction, 117 milligrams of crude material were obtained. This was fractionally distilled at reduced pressures and yielded two distinct fractions. The first, 6 milligrams distilling at 150°–152° C. and 21 mm., was a clear liquid. Analysis showed it to have a molecular weight of 156, to contain one oxygen atom and to have a carbon-hydrogen ratio of 1 : 2. The second fraction, 42 milligrams distilling at 190°–200° C. at 3.5 mm., was a waxy solid melting slightly above room temperature. Analysis showed it to have a carbon-hydrogen ratio of 1 : 2, to contain only 2.5% oxygen, and to have a molecular weight of 464. Since the complete separation of such small quantities of a mixture by a single fractional distillation is not possible, it is certain that each fraction was contaminated with some of the other. Accordingly, the high boiling substance may be a hydrocarbon and the low boiling substance an oxygen containing compound of still lower molecular weight. With the material at hand it was impossible to further separate these mixtures by repeated fractionation. The second fraction did not appear to be the odoriferous compound sought as small amounts, on exposure to air, soon lost all traces of the odor, apparently held temporarily by absorption. The first fraction possessed a permanently strong odor.

The yield from such a large amount of pressed oil was much lower than might have been expected from the results of the trial run using extracted oil. One must therefore conclude that the oil extracted by means of ether contains much more of the odorous compound. To

establish this, 600 grams each of pressed oil, extracted oil from whole corn, and extracted oil from yellow corn germ, were steam-distilled until 1 liter of condensate was collected from each portion and the odors then qualitatively compared. The condensates from the two portions of extracted oil were apparently equal in pungency while that from the pressed oil possessed much less odor. There are two possible explanations for this. The odorizing matrial may be held in the germ in such a manner that the pressing out of the oil does not carry the odorizer with it. On the other hand the temperature at which the pressing process is carried out may be sufficient to distill out a portion of the odoriferous material. At any rate, the amount of the compound present in yellow corn appears to be surprisingly low. Extractions were carried out on white corn but the resulting oils possessed so little odor that no attempt was made to isolate the odorous compounds.

The authors wish to express their appreciation of the kindness of the Miller Cereal Mills of Omaha in supplying them with materials.

## Summary

A volatile compound having the characteristic odor of yellow corn has been isolated from yellow corn oil.

It is a compound with a molecular weight of less than 150 and contains one atom of oxygen.

The yield was approximately one part per million parts of pressed oil.

# A COMPARISON BETWEEN THE STANDARD BASIC AND MALT-PHOSPHATE-BROMATE BAKING METHODS ON 1937 NORTH DAKOTA HARD RED SPRING WHEAT

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## Introduction

The problem of evaluating the inherent quality of wheat flour has been widely discussed by cereal chemists, and a variety of baking methods has been suggested for accomplishing this purpose. One flour may vary so widely in its response to different baking procedures that it becomes increasingly difficult for the technologist to place properly the flour in regard to baking quality. It is probable that the term "potential baking quality" would be of more general utility as suggested by Cayzer (1936), as it is an absolute expression, and may therefore be used with less reserve. As used by Cayzer, this term signifies the ability of a flour to yield satisfactory baking results under severe conditions of baking procedure. The higher the "potential baking quality" of a flour the larger the loaf produced as these conditions are made more drastic. It must be borne in mind, however, that the imposition of too severe conditions will reduce the loaf volume of high protein flours to a greater degree than those of lower protein content, as postulated by Aitken and Geddes (1934).

Geddes and Larmour (1933) concluded that the inclusion of potassium bromate in the baking formula led to a better differentiation of baking quality in Western Canadian hard red spring wheat flours. The correlation of loaf volume with protein content was higher with the bromate than when the basic formula was used, and the bromate method was more sensitive in bringing out modifications in baking quality. The basic method was influenced more by variation in the enzymic activity of the flour. In the higher protein ranges larger increments of bromate would probably be required to bring out the full potentiality of a flour. Larmour, Geddes, and Whiteside (1933) found the malt-phosphate-bromate formula to give the greatest range of loaf volume when testing various hybrids and standard varieties for Canadian plant breeders. The effect of diastatic activity appeared to be ruled out by this baking procedure. The authors did not con-

clude that the malt-phosphate-bromate formula was superior to the bromate formula for the type of work cited.

Blish (1934) cited the utility of the basic procedure of the Standard A. A. C. C. baking test as a point of reference. Other and supplementary procedures could be used to obtain additional information regarding flour quality.

Aitken and Geddes (*loc. cit.*) found that the malt-phosphate-bromate formula with a 3-hour fermentation period yielded the largest absolute loaf volumes, as well as maximum differentiation, when 10 composite wheat samples obtained by blending the surpluses of samples from the 1932 protein survey of Western Canadian hard red spring wheat were baked by a variety of methods. By imposing severe baking conditions the loaf volume of the higher protein flours was reduced to a greater degree than those of lower protein content, thus decreasing the differences between the flours. Loaf volume alone was considered to be an index of baking quality in this investigation.

In a study of various methods of evaluating baking quality, Kent-Jones and Geddes (1936) employed a number of formulas, including the basic, malt-phosphate, malt-phosphate-bromate, and malt-phosphate-bromate with variable mixing time. A wide range of baking quality existed in the series of flours used, and it was found that the malt-phosphate-bromate formula with 3-minute mixing time in a Hobart-Swanson mixer yielded maximum differentiation among the flours. The malt-phosphate-bromate formula with the usual (1-minute) mixing time ranked second in differentiating the different samples.

The application of the bromate supplement to North Dakota hard red spring wheat has not always appeared to yield satisfactory results in the form of increased loaf volume and better differentiation when compared with the standard basic method. This fact has not been in accord with the author's experience in working with hard red spring wheats grown in Western Canada, or with several samples previously tested of American grown wheat. It did not seem probable that a difference of a few miles in distance would cause such marked differences in the environment as would alter to any appreciable extent a fundamental attribute of wheat such as positive response to a properly balanced baking formula. In view of these considerations it was considered desirable to study the effect of the malt-phosphate-bromate formula in comparison with the standard basic procedure upon the current crop of North Dakota hard red spring wheat.

## Experimental

### Material and Methods

A series of 89 samples of hard red spring wheat grown in the State of North Dakota during the crop season of 1937 were experimentally

TABLE I

COMPARATIVE GRADES AND ANALYTICAL DATA OF SAMPLES ARRANGED IN ORDER OF INCREASING WHEAT PROTEIN CONTENT [1]

| Laboratory number | Grade [2] | Crude protein (N × 5.7) | | Moisture | Ash | Diastatic activity mg. maltose per 10 g. flour |
|---|---|---|---|---|---|---|
| | | Wheat | Flour | | | |
| | | % | % | % | % | |
| 37–8–31 | 4 DNS | 11.1 | 10.3 | 14.0 | 0.50 | 146.0 |
| 44 | 1 DNS | 11.4 | 10.7 | 14.2 | .46 | 140.7 |
| 27 | 4 DNS | 12.1 | 11.3 | 14.7 | .46 | 131.8 |
| 25 | 3 DNS | 12.2 | 11.6 | 13.9 | .48 | 158.2 |
| 35 | 4 DNS | 12.3 | 11.8 | 14.7 | .54 | 154.4 |
| 37–9–38 | S DNS | 12.3 | 11.6 | 14.3 | .52 | 106.0 |
| 37–8–15 | 1 NS | 12.4 | 11.5 | 14.1 | .47 | 119.4 |
| 36 | 1 DNS | 12.4 | 11.9 | 14.5 | .47 | 129.0 |
| 37–9–40 | S DNS | 12.5 | 12.1 | 13.5 | .57 | 167.0 |
| 37–8–33 | S DNS | 12.9 | 12.1 | 14.6 | .58 | 126.5 |
| 43 | 5 DNS | 12.9 | 12.0 | 14.2 | .49 | 101.5 |
| 37–9–30 | 2 DNS | 12.9 | 12.4 | 14.3 | .48 | 151.4 |
| 29 | 3 DNS | 13.0 | 12.5 | 14.2 | .48 | 167.1 |
| 33 | 3 DNS | 13.0 | 12.4 | 14.0 | .50 | 158.6 |
| 37–8–34 | 1 DNS | 13.2 | 12.1 | 14.9 | .51 | 126.6 |
| 37–9– 2 | 2 DNS | 13.2 | 12.7 | 14.4 | .47 | 121.4 |
| 15 | S DNS | 13.2 | 12.1 | 13.5 | .61 | 177.0 |
| 37–8– 5 | 1 HvRS | 13.3 | 12.2 | 13.9 | .45 | 137.0 |
| 38 | 1 DNS | 13.4 | 12.9 | 14.3 | .46 | 123.5 |
| 37–9–14 | S DNS | 13.5 | 12.6 | 13.5 | .63 | 174.2 |
| 37–8–20 | 2 DNS | 13.6 | 13.2 | 13.6 | .46 | 135.5 |
| 51 | S DNS | 13.6 | 12.3 | 12.3 | .51 | 106.0 |
| 37–9–21 | 4 DNS | 13.6 | 12.7 | 14.4 | .50 | 150.0 |
| 37–8–42 | 5 DNS | 13.8 | 13.3 | 14.3 | .41 | 105.0 |
| 37–9– 4 | S DNS | 13.8 | 13.2 | 14.2 | .56 | 123.5 |
| 20 | 1 DNS | 13.8 | 13.2 | 13.6 | .46 | 135.5 |
| 22 | S DNS | 13.9 | 12.9 | 13.9 | .62 | 136.0 |
| 31 | S DNS | 13.9 | 13.3 | 13.9 | .50 | 123.7 |
| 37–8–26 | 4 DNS | 14.0 | 13.3 | 13.8 | .44 | 144.4 |
| 37–9– 5 | 1 HvNS | 14.0 | 13.7 | 14.6 | .45 | 91.2 |
| 32 | S DNS | 14.0 | 13.2 | 14.2 | .55 | 145.4 |
| 37–8– 8 | S DNS | 14.1 | 13.0 | 14.2 | .55 | 146.0 |
| 50 | 5 DNS | 14.1 | 13.8 | 13.0 | .51 | 97.0 |
| 47 | S DNS | 14.2 | 13.1 | 14.1 | .54 | 148.0 |
| 37–9– 6 | S DNS | 14.2 | 13.8 | 14.7 | .51 | 114.8 |
| 34 | S DNS | 14.2 | 13.3 | 14.1 | .47 | 106.5 |
| 1 | S DNS | 14.3 | 13.2 | 14.0 | .58 | 120.4 |
| 28 | 3 DNS | 14.3 | 13.7 | 14.1 | .49 | 121.0 |
| 37–8–11 | 3 DNS | 14.4 | 13.9 | 14.7 | .49 | 129.0 |
| 21 | 5 DNS | 14.4 | 13.8 | 14.4 | .65 | 137.0 |
| 37 | S DNS | 14.4 | 13.8 | 14.8 | .59 | 99.0 |
| 37–9–12 | S NS | 14.4 | 13.4 | 13.4 | .60 | 147.3 |
| 16 | 4 DNS | 14.4 | 14.0 | 14.6 | .53 | 99.4 |
| 24 | 5 DNS | 14.4 | 13.2 | 14.4 | .72 | 100.0 |
| 37–9–25 | 4 DNS | 14.4 | 13.7 | 14.8 | .47 | 98.0 |
| 37–8– 9 | 3 DNS | 14.5 | 13.9 | 13.4 | .52 | 158.2 |
| 30 | 2 DNS | 14.5 | 13.8 | 13.3 | .56 | 162.0 |

TABLE I—*Continued*

| Laboratory number | Grade [2] | Crude protein (N × 5.7) | | Moisture | Ash | Diastatic activity mg. maltose per 10 g. flour |
|---|---|---|---|---|---|---|
| | | Wheat | Flour | | | |
| | | % | % | % | % | |
| 37–9–18 | 4 DNS | 14.5 | 13.6 | 14.8 | 0.46 | 98.7 |
| 26 | S DNS | 14.5 | 13.6 | 14.5 | .54 | 114.5 |
| 37–8– 4 | 5 NS | 14.6 | 14.0 | 14.3 | .46 | 88.7 |
| 18 | 4 NS | 14.6 | 14.2 | 14.5 | .52 | 118.0 |
| 28 | S DNS | 14.6 | 13.3 | 13.8 | .57 | 140.7 |
| 37–9–10 | S NS | 14.6 | 14.0 | 14.1 | .49 | 113.4 |
| 23 | 3 DNS | 14.6 | 13.8 | 13.9 | .50 | 138.0 |
| 37 | S DNS | 14.6 | 13.8 | 14.1 | .49 | 110.8 |
| 37–8–45 | 4 DNS | 14.7 | 14.2 | 14.6 | .41 | 103.3 |
| 48 | 3 DNS | 14.8 | 14.1 | 13.7 | .48 | 142.0 |
| 37–9–36 | S NS | 14.8 | 13.8 | 13.4 | .52 | 126.0 |
| 37–8–41 | 5 DNS | 14.9 | 14.1 | 14.2 | .54 | 133.2 |
| 46 | 2 DNS | 14.9 | 14.6 | 14.3 | .47 | 110.0 |
| 29 | 4 DNS | 15.0 | 14.4 | 14.4 | .46 | 102.4 |
| 12 | 5 DNS | 15.1 | 13.8 | 14.2 | .56 | 131.0 |
| 37–9–11 | S NS | 15.1 | 14.4 | 14.0 | .62 | 142.0 |
| 35 | S DNS | 15.1 | 14.5 | 13.4 | .50 | 122.5 |
| 37–8– 1 | 5 NS | 15.2 | 14.1 | 15.2 | .50 | 82.0 |
| 37–9– 9 | S DNS | 15.2 | 14.6 | 14.1 | .54 | 94.0 |
| 37–8–14 | 1 DNS | 15.4 | 14.6 | 13.9 | .43 | 125.5 |
| 22 | 3 DNS | 15.4 | 14.9 | 14.2 | .65 | 164.5 |
| 37–9–27 | S DNS | 15.4 | 14.4 | 13.9 | .58 | 125.5 |
| 37–8–19 | S NS | 15.5 | 14.7 | 13.9 | .59 | 113.5 |
| 40 | S DNS | 15.5 | 14.8 | 14.3 | .56 | 121.8 |
| 37–9– 3 | S DNS | 15.6 | 15.2 | 14.2 | .49 | 106.4 |
| 39 | 3 DNS | 15.6 | 15.0 | 13.6 | .53 | 153.0 |
| 37–8– 7 | 5 NS | 15.7 | 15.0 | 14.0 | .48 | 83.7 |
| 3 | 4 NS | 15.8 | 15.0 | 14.3 | .44 | 88.9 |
| 2 | S NS | 16.0 | 15.4 | 14.1 | .55 | 115.6 |
| 32 | 4 DNS | 16.0 | 15.4 | 13.8 | .59 | 125.4 |
| 37–9– 8 | 3 DNS | 16.1 | 15.7 | 14.2 | .50 | 100.8 |
| 37–8–13 | 5 DNS | 16.3 | 15.4 | 13.6 | .52 | 104.9 |
| 37–9 –7 | 1 DNS | 16.3 | 15.4 | 14.5 | .51 | 102.0 |
| 37–8–23 | 5 DNS | 16.4 | 16.3 | 13.8 | .72 | 165.6 |
| 37–8–39 | 5 DNS | 16.5 | 16.2 | 14.1 | .49 | 80.0 |
| 37–8–24 | 5 DNS | 17.0 | 15.6 | 13.4 | .67 | 224.7 |
| 17 | S NS | 17.1 | 16.2 | 13.3 | .95 | 132.4 |
| 37–9–13 | S NS | 17.5 | 16.0 | 13.1 | .76 | 210.3 |
| 37–8–49 | 5 DNS | 17.7 | 17.2 | 13.8 | .49 | 85.7 |
| 6 | S NS | 18.0 | 17.0 | 14.0 | .44 | 76.0 |
| 10 | S DNS | 19.0 | 17.6 | 14.5 | .51 | 74.8 |
| 16 | S NS | 19.3 | 19.1 | 14.0 | .49 | 102.6 |

[1] Analytical results calculated to 13.5% moisture basis.
[2] Unofficial.

milled into straight flour on an Allis-Chalmers mill. These wheats were all free from damage by bleaching or weathering but some had been affected by rust and heat. An effort was made to secure an even distribution of the samples from the different counties in the State, but owing to drought conditions in the Western portion no samples

TABLE II

COMPARATIVE RESULTS OBTAINED BY THE TWO BAKING METHODS ARRANGED IN ORDER OF INCREASING WHEAT PROTEIN CONTENT

| Laboratory number | Absorption | Baking procedure | | | | | |
|---|---|---|---|---|---|---|---|
| | | Standard | | | Malt-phosphate-bromate [1] | | |
| | | Loaf volume | Color | Texture | Loaf volume | Color | Texture |
| | | c.c. | | | c.c. | | |
| 37-8-31 | 60 | 476 | 94 | 95 | 556 | 94 | 94 |
| 44 | 60 | 495 | 94 | 95 | 581 | 94 | 93.5 |
| 27 | 61 | 505 | 94 | 93 | 570 | 95 | 94 |
| 25 | 60 | 500 | 92 | 93 | 655 | 95 | 93 |
| 35 | 61 | 510 | 92 | 93 | 641 | 93 | 93 |
| 37-9-38 | 64 | 533 | 94 | 94 | 580 | 93 | 92 |
| 37-8-15 | 59 | 511 | 92 | 94 | 550 | 94 | 94 |
| 36 | 62 | 502 | 93 | 93 | 612 | 94 | 93 |
| 37-9-40 | 65 | 529 | 92 | 94 | 661 | 91 | 92 |
| 37-8-33 | 60 | 553 | 92 | 93 | 698 | 93 | 92 |
| 43 | 61 | 522 | 92 | 95 | 660 | 93 | 93 |
| 37-9-30 | 64 | 544 | 93 | 92.5 | 630 | 94 | 91 |
| 29 | 63 | 551 | 94 | 93 | 636 | 93 | 91 |
| 33 | 60 | 545 | 92 | 92 | 694 | 94 | 92 |
| 37-8-34 | 62 | 502 | 93 | 93 | 575 | 93 | 92 |
| 37-9- 2 | 62 | 515 | 94 | 94 | 675 | 94 | 91 |
| 15 | 65 | 545 | 92 | 92 | 652 | 93 | 93 |
| 37-8- 5 | 61 | 546 | 93 | 94 | 588 | 94 | 94 |
| 38 | 60 | 542 | 93 | 93 | 653 | 94 | 93 |
| 37-9-14 | 64 | 545 | 92 | 94 | 698 | 94 | 93 |
| 37-8-20 | 60 | 533 | 92 | 94 | 711 | 95 | 93 |
| 51 | 63 | 560 | 92 | 92 | 712 | 93 | 93 |
| 37-9-21 | 63 | 591 | 95 | 94 | 662 | 93 | 93 |
| 37-8-42 | 61 | 544 | 94 | 94 | 650 | 94 | 93 |
| 37-9- 4 | 62 | 568 | 92 | 94 | 665 | 92 | 93 |
| 20 | 62 | 577 | 94 | 94 | 710 | 94 | 92 |
| 22 | 63 | 555 | 93 | 92 | 700 | 93 | 91 |
| 31 | 63 | 556 | 92 | 94 | 762 | 93 | 91 |
| 37-8-26 | 60 | 542 | 94 | 95 | 655 | 95 | 94 |
| 37-9- 5 | 60 | 515 | 94 | 94 | 677 | 95 | 93 |
| 32 | 62 | 573 | 93 | 94 | 675 | 93 | 92 |
| 37-8- 8 | 61 | 584 | 92 | 94 | 631 | 93 | 93 |
| 50 | 63 | 556 | 92 | 94 | 752 | 93 | 92 |
| 47 | 63 | 565 | 92 | 92 | 720 | 93 | 93 |
| 37-9- 6 | 61 | 596 | 94 | 92 | 669 | 92 | 91 |
| 34 | 63 | 554 | 94 | 92 | 724 | 94 | 92 |
| 1 | 62 | 528 | 93 | 94 | 625 | 94 | 93 |
| 28 | 61 | 548 | 94 | 92 | 735 | 94 | 91.5 |
| 37-8-11 | 62 | 564 | 92 | 94 | 695 | 94 | 90.5 |
| 21 | 60 | 588 | 93 | 92 | 706 | 94 | 94 |
| 37 | 60 | 596 | 92 | 92 | 662 | 92 | 93 |
| 37-9-12 | 63 | 543 | 94 | 93.5 | 737 | 94 | 93 |
| 16 | 60 | 530 | 92 | 92 | 716 | 93 | 92 |
| 24 | 65 | 548 | 92 | 92 | 700 | 94 | 91 |

TABLE II—*Continued*

| Laboratory number | Absorption | Baking procedure | | | | | |
|---|---|---|---|---|---|---|---|
| | | Standard | | | Malt-phosphate-bromate [1] | | |
| | | Loaf volume | Color | Texture | Loaf volume | Color | Texture |
| | | *c.c.* | | | *c.c.* | | |
| 37–9–25 | 61 | 545 | 93 | 94 | 743 | 94 | 90.5 |
| 37–8– 9 | 62 | 553 | 90 | 94 | 707 | 94 | 92 |
| 30 | 62 | 582 | 94 | 92 | 664 | 93 | 94 |
| 37–9–18 | 62 | 532 | 93 | 92 | 795 | 93 | 92 |
| 26 | 63 | 549 | 93 | 94 | 743 | 94 | 92 |
| 37–8– 4 | 60 | 540 | 92 | 94.5 | 725 | 93 | 92 |
| 18 | 60 | 572 | 93 | 92 | 730 | 95 | 92 |
| 28 | 62 | 634 | 93 | 92 | 677 | 92 | 94 |
| 37–9–10 | 62 | 539 | 93 | 93.5 | 700 | 94 | 93 |
| 23 | 63 | 557 | 94 | 94 | 817 | 94 | 91 |
| 37 | 64 | 569 | 94 | 92 | 737 | 92 | 91 |
| 37–8–45 | 62 | 511 | 92 | 94.5 | 745 | 93 | 91 |
| 48 | 63 | 582 | 92 | 94 | 745 | 94 | 93 |
| 37–9–36 | 63 | 563 | 93 | 92 | 735 | 92 | 91 |
| 37–8–41 | 61 | 526 | 94 | 94 | 668 | 94 | 93 |
| 46 | 62 | 560 | 94 | 94 | 768 | 93 | 91 |
| 29 | 62 | 537 | 94 | 95 | 778 | 93 | 92 |
| 12 | 61 | 608 | 90 | 94 | 743 | 92 | 91 |
| 37–9–11 | 62 | 592 | 92 | 94 | 810 | 94 | 93 |
| 35 | 63 | 601 | 95 | 92 | 860 | 92 | 91 |
| 37–8– 1 | 59 | 548 | 92 | 92 | 658 | 91 | 91.5 |
| 37–9– 9 | 63 | 548 | 92 | 92 | 758 | 93 | 91 |
| 37–8–14 | 62 | 598 | 94 | 93.5 | 730 | 94 | 92 |
| 22 | 60 | 616 | 93 | 92 | 800 | 93 | 93 |
| 37–9–27 | 62 | 604 | 91 | 93 | 820 | 92 | 92.5 |
| 37–8–19 | 62 | 528 | 88 | 94 | 703 | 92 | 92 |
| 40 | 60 | 578 | 92 | 92 | 672 | 92 | 91 |
| 37–9– 3 | 62 | 545 | 94 | 92 | 830 | 95 | 93 |
| 39 | 65 | 629 | 95 | 94 | 780 | 92 | 91 |
| 37–8– 7 | 60 | 521 | 93 | 93 | 768 | 95 | 92 |
| 3 | 60 | 550 | 93 | 94 | 852 | 95 | 90 |
| 2 | 60 | 578 | 90 | 95 | 690 | 92 | 92 |
| 32 | 61 | 557 | 94 | 92 | 758 | 93 | 92 |
| 37–9– 8 | 62 | 591 | 94 | 92 | 818 | 93 | 90 |
| 37–8–13 | 61 | 600 | 93 | 92 | 715 | 93 | 92 |
| 37–9– 7 | 61 | 561 | 94 | 93 | 788 | 93 | 90 |
| 37–8–23 | 60 | 550 | 91 | 93 | 758 | 93 | 90.5 |
| 37–8–39 | 61 | 562 | 92 | 92 | 840 | 93 | 92.5 |
| 37–8–24 | 61 | 632 | 91 | 92 | 825 | 93 | 90 |
| 17 | 60 | 645 | 84 | 93 | 880 | 90 | 91 |
| 37–9–13 | 62 | 622 | 91 | 92 | 817 | 92 | 92.5 |
| 37–8–49 | 63 | 562 | 91 | 92 | 825 | 93 | 91 |
| 6 | 60 | 556 | 93 | 93 | 933 | 93 | 90 |
| 10 | 60 | 563 | 90 | 93 | 793 | 93 | 92 |
| 16 | 60 | 661 | 93 | 92 | 1070 | 93 | 92 |

[1] 0.3% 60° Lintner malt, 0.1% ammonium phosphate and 0.001% $KBrO_3$.

were obtained from several counties. The samples were cleaned from dockage and scoured before milling, then tempered to a moisture content of 15% for 20 hours prior to milling.

The protein content of the wheat and flour was determined, also flour ash, moisture, and diastatic activity. The flours were then baked by two methods—the standard basic and the malt-phosphate-bromate formulas.[1] Both formulas had 5% of sucrose included instead of the usual 3%.

## The Data

The wheat grades, crude wheat and flour protein, ash, moisture and diastatic activity are shown in Table I. The items are arranged in order of increasing wheat protein content, as this variable is commonly considered to be an index of baking quality. A scrutiny of the data shows that wheat and flour protein are very closely associated, the flour protein running lower than the corresponding wheat protein due to the removal in the milling process of the branny layer with its attendant high protein content. A large variability in wheat grades is evident, reflecting the variation in the environment and rust incidence among the samples. The ash is somewhat variable but does not appear to be closely associated with the grade among the different flours.

The baking results obtained, in terms of loaf volume, color, and texture, are shown in Table II, arranged as before in order of increasing crude wheat protein content. When loaf volumes obtained by the two baking methods are compared, an increase in loaf volume with the malt-phosphate-bromate method is noticeable in every instance. This positive response varies from 39 to 409 c.c., and is especially evident among the higher protein flours. The color of the loaves was increased by the malt-phosphate-bromate formula, but the texture was coarser owing principally to increased size of the loaf with consequent gas pockets in the interior. The flour milled from the lower grades appeared to average the same in absorption as the flour from the better grades. While the color of the crumb does not appear to change materially with increase in wheat protein, the texture score of the loaves baked by the malt-phosphate-bromate method tends to decrease with increasing protein. This is caused by the greater expansion produced by this formula with high protein flour.

In Table III are shown the responses to the malt-phosphate-bromate formula in cubic centimeters of difference between the loaf volume obtained by the two methods. As the responses were all positive, no algebraical sign was included in the table. A perusal of

[1] 0.3% 60° Lintner malt, 0.1% ammonium phosphate and 0.001% $KBrO_3$.

the data reveals increased response as the protein content increases. This again would be expected because of the larger loaves obtained by the use of the malt-phosphate-bromate formula. This response to an improver has been suggested as a criterion of flour strength because it measures the increase in loaf size produced under the stimulus of the improver. Here the standard basic loaf volume is used as a point of reference, as proposed by Blish (*loc. cit.*).

TABLE III

RESPONSE TO MALT-PHOSPHATE-BROMATE FORMULA

| Laboratory number | C.c. | Laboratory number | C.c. | Laboratory number | C.c. |
|---|---|---|---|---|---|
| 37-8-31 | 80 | 37-9-32 | 102 | 37-8-29 | 241 |
| 44 | 86 | 37-8- 8 | 47 | 12 | 135 |
| 27 | 65 | 50 | 196 | 37-9-11 | 218 |
| 25 | 155 | 47 | 155 | 35 | 259 |
| 35 | 131 | 37-9- 6 | 73 | 37-8- 1 | 110 |
| 37-9-38 | 47 | 34 | 170 | 37-9- 9 | 210 |
| 37-8-15 | 39 | 1 | 97 | 37-8-14 | 132 |
| 36 | 110 | 28 | 187 | 22 | 184 |
| 37-9-40 | 132 | 37-8-11 | 131 | 37-9-27 | 216 |
| 37-8-33 | 145 | 21 | 118 | 37-8-19 | 175 |
| 43 | 138 | 37 | 66 | 40 | 94 |
| 37-9-30 | 86 | 37-9-12 | 194 | 37-9- 3 | 285 |
| 29 | 85 | 16 | 186 | 39 | 151 |
| 33 | 149 | 24 | 152 | 37-8- 7 | 247 |
| 37-8-34 | 73 | 25 | 198 | 3 | 302 |
| 37-9- 2 | 160 | 37-8- 9 | 154 | 2 | 112 |
| 15 | 107 | 30 | 82 | 32 | 201 |
| 37-8- 5 | 42 | 37-9-18 | 263 | 37-9- 8 | 227 |
| 38 | 111 | 26 | 194 | 37-8-13 | 115 |
| 37-9-14 | 153 | 37-8- 4 | 185 | 37-9- 7 | 227 |
| 37-8-20 | 178 | 18 | 158 | 37-8-23 | 208 |
| 51 | 152 | 28 | 43 | 37-8-39 | 278 |
| 37-9-21 | 71 | 37-9-10 | 161 | 37-8-24 | 193 |
| 37-8-42 | 106 | 23 | 260 | 17 | 235 |
| 37-9- 4 | 97 | 37 | 168 | 37-9-13 | 195 |
| 20 | 133 | 45 | 234 | 37-8-49 | 263 |
| 22 | 145 | 37-8-48 | 163 | 6 | 377 |
| 31 | 206 | 37-9-36 | 172 | 10 | 230 |
| 37-8-26 | 113 | 37-8-41 | 142 | 16 | 409 |
| 37-9- 5 | 162 | 46 | 208 | | |

The correlation coefficients and other statistical constants are grouped in Table IV. The mean protein content between wheat and flour shows a difference of only 0.74% for all the samples included in this study. The flour protein tends to be slightly more variable than the wheat protein and this can probably be explained by small variations in the milling technique. The malt-phosphate-bromate (*B*) baking method produced loaves which averaged 161 c.c. higher than the standard basic (*A*) method. The malt-phosphate-bromate method also gave better differentiation between the samples. All the corre-

lation constants tabulated are very significant. Flour protein could evidently be predicted from the knowledge of wheat protein in material of this nature. The relationship between these variables is not entirely constant, however, as pointed out by Waldron and Mangels (1932). These workers found the lowest correlation constant between these characters to exist in the instance of nursery samples where diverse strains or varieties are included. Flour protein and loaf volume were more highly related when baking method (*B*) was employed.

TABLE IV

TABLE OF STATISTICAL CONSTANTS

Means, Standard Deviations and Coefficients of Variability

| | Means | Standard deviation | Coefficient of variability |
|---|---|---|---|
| Wheat protein | 14.55 | 1.51 | 10.38 |
| Flour protein | 13.81 | 1.51 | 10.93 |
| Ash | 0.526 | 0.083 | 15.78 |
| Diastatic activity | 125.57 | 22.824 | 18.18 |
| Loaf volume (A) | 557.77 | 35.04 | 6.28 |
| Loaf volume (B) | 718.95 | 84.99 | 11.82 |

SIMPLE CORRELATION COEFFICIENTS

| Variables correlated | | | |
|---|---|---|---|
| *X* | *Y* | $r_{xy}$ | *P* [1] |
| Flour protein | Loaf volume (A) [2] | + .5892 | < .0001 |
| Flour protein | Loaf volume (B) [3] | + .8361 | < .0001 |
| Flour protein | Wheat protein | + .9818 | < .0001 |
| Loaf volume (A) [2] | Loaf volume (B) [3] | + .5733 | < .0001 |

[1] P = probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.
[2] A = Standard baking method.
[3] B = Malt-phosphate-bromate method.

The significance of the differences between the correlation coefficients computed between flour protein and the loaf volume data is shown in Table V. Fisher's (1928) method of transformation of the correlation coefficient to the *Z* value was used. The significance of the difference may be computed by finding the relative deviate $X^1$ from the ratio of the difference in *Z* value $(Z_1 - Z_2)$ to its standard deviation $\left(\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}}\right)$, $N_1$ and $N_2$ in the present instance being 89. From a knowledge of the relative deviate the probability of the observed difference occurring owing to the forces of chance incidence may be evaluated. It is apparent that there is a significant

difference between the correlation coefficients obtained between flour protein and loaf volumes yielded by the two baking methods.

TABLE V

TESTS OF SIGNIFICANCE OF DIFFERENCE BETWEEN CORRELATION COEFFICIENTS COMPUTED FROM FLOUR PROTEIN AND LOAF VOLUME DATA

| Variable Correlated with Flour Protein | Correlation | $Zr$ | $d/\sigma d$ | $X^1$ | $P$ |
|---|---|---|---|---|---|
| Formula (B) | .8361 | 1.2083 | $\frac{.5320}{\sqrt{1/43}}$ | | |
| Formula (A) | .5892 | 0.6763 | | | |
| difference ($d$) | | 0.5320 | | 3.488 | .0005 |

## Discussion of Results

The malt-phosphate-bromate formula with 2-minute mixing time in the Hobart-Swanson gave very satisfactory results with the samples tested from the 1937 crop of North Dakota hard red spring wheat. Higher loaf volumes were registered by its use, and the crumb color was improved when compared with the corresponding loaves baked by the standard basic method. These results are characteristic of wheats possessing high inherent quality and such wheats and the flours milled therefrom should accordingly be suitable for blending with weaker and "softer" wheats to improve the "potential baking quality" of the blends.

From the data included in this investigation it would seem that the standard basic method should be used in conjunction with a second method, or possibly more methods, which would tend to bring out the inherent possibility of a flour, or the "potential baking quality," using the term suggested by Cayzer (*loc. cit.*).

In a study of this nature flour protein could be estimated with sufficient accuracy for practical purposes from a knowledge of wheat protein. Similarly, wheat protein could be estimated from a knowledge of flour protein. The statistical constants are shown in Table VI.

TABLE VI

LINEAR REGRESSION COEFFICIENTS

Flour protein $\%^{(y)}$ on wheat protein $\%^{(x)}$, $b = .9827$

Wheat protein $\%^{(x)}$ on flour protein $\%^{(y)}$, $b^1 = .9809$

LINEAR REGRESSION EQUATIONS

Estimation of flour protein $\%^{(y)}$ from wheat protein $\%^{(x)}$

$Y = -.4827 + .9827x$

Error of estimate = .29%

Estimation of wheat protein $\%^{(x)}$ from flour protein $\%^{(y)}$

$X = +.9983 + .9809y$

Error of estimate = .29%

## Summary and Conclusions

A straight flour was milled from 89 samples of North Dakota hard red spring wheat of the 1937 crop. Analytical determinations were run upon the wheat and flour, and the flours were baked by two methods—the standard basic with 5% sucrose, and the malt-phosphate-bromate. Response in cubic centimeters to the latter formula was calculated.

An examination of the data revealed an extremely high positive correlation between wheat and flour protein. This relationship could be used for predicting one variable from a knowledge of the other. The loaf volumes obtained by both methods of baking were significantly and positively correlated with the flour protein.

It was shown that the malt-phosphate-bromate method gave a significantly higher correlation with flour protein than the standard basic procedure.

North Dakota hard red spring wheat flour was shown to respond to the malt-phosphate-bromate formula in a satisfactory manner, yielding larger loaves of improved color, and giving better differentiation among the various samples. The higher protein flours gave loaves of coarser texture than the low protein samples.

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# THE RELATION OF FLOUR STRENGTH, SOY FLOUR AND TEMPERATURE OF STORAGE TO THE STALING OF BREAD [1]

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(Read at the Annual Meeting, May 1937)

As wheat flour bread ages several distinct changes in its properties take place. These commonly include loss of water, loss of flavor and aroma, crust staling and crumb staling. The changes which have been found to occur in crumb staling include the lowering of the absorptive power of the starch, the decrease in the content of water-soluble amylose, the hardening of the crumb, the development of crumbliness and the accompanying change in the X-ray pattern of the starch.

As early as 1853 Boussingault distinguished between the hardening of bread due to the evaporation of water and that due to inherent or true staling as known today. He demonstrated that true staling is the more important and took place even when evaporation was prevented by storing the bread in hermetically sealed containers.

Horsford (1873) was apparently the first to suggest that the staling of bread is due to a change in the distribution of water between the starch and gluten. Lindet (1902) attributed staleness to what he called the "retrogradation" of starch. Katz (1913) proposed the hypothesis that there exists in the crumb of bread a physico-chemical equilibrium involving the state of the starch and believed that the rate at which staleness sets in is dependent on temperature. He found that at from 50° to 100° C. fresh bread is the equilibrium state and at from 25° to 0° C. stale bread is the stable form. He found no definite temperature at which bread became stale but noted the most rapid increase in the rate of onset of staleness between 50° and 25° C. Recently Alsberg (1936) has explained that as bread cools the soft starch jelly sets into a stiff but still elastic gel which contains less water than is necessary for complete gelatinization. The starch gel shrinks a little during the process of setting and for some time afterward. Because the gluten is losing moisture to either the starch or crumb solution, or both, it may also shrink and the strains which are set up at the gluten-starch interfaces ultimately cause them to separate and the bread becomes crumbly.

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Platt (1930) confirmed Katz's observations upon the effect of temperature on bread staling and his experiments also indicated that lean formula bread became stale more rapidly than did rich formula bread. His bread made from the rich formula not only remained softer throughout the test but the richer bread had a slightly but significantly slower rate of staling than the lean bread. In an experiment conducted by Whymper (1919) it was found that, judged organoleptically, bread made from a dough to which extra gluten had been added had better keeping qualities than bread made from the regular dough.

## Experimental

Two types of bread were employed in all of the staling studies. The regular bread was made with 100% flour, 1½% salt, 2½% shortening, 3% sugar, 5% yeast and (variable) water. The second type of bread included in addition to the ingredients listed above, 1½% defatted soybean flour and 4½% additional water. These two types of bread were given identical treatment during fermentation, baking and storage. The dough batches were of such size that all loaves of a particular type used in a single study were obtained from the same dough. The doughs were fermented for one hour at 30° C. with one kneading. After 35 minutes' pan proof at 30° C. the loaves were baked for 30 minutes at 230° C.

After cooling one hour at room temperature, measurements were made on one loaf of each type of bread and the remaining loaves were wrapped and sealed in wax paper and placed in storage. Fresh bread measurements were made on one loaf from each batch.

In this study three methods of measuring staleness were employed concurrently to follow the changes in the aging of bread crumb. A measure of the firmness of the crumb was made with an apparatus similar to that used by Platt (1930). This device measures the depression of the surface of a slice of bread crumb effected by a plunger acting under a unit force or weight. The second method was that of Katz (1928) and involved the determination of swelling power by measuring the volume of sediment obtained from 10 grams of finely ground bread crumb. The crumb of fresh bread occupies a greater volume than that of stale bread due to the greater water-absorbing capacity of the fresh bread starch. The third test employed Karacsonyi's (1929) method of measuring the viscosity of a 10% suspension of finely ground bread crumb. The use of this test as a measurement of staleness is also based on the swelling power of the starch.

Throughout this study the results which are shown are the average values of staling tests conducted on replicated loaves. After storage under the conditions which will be specified, the tests were repeated on

the aged bread, and the percentage reduction of these values from the fresh bread values were plotted against time of storage. The curves having the greatest slope and reaching the highest values thus represent the bread which has undergone the greatest amount of staling.

### The Effect of Flour Strength on the Keeping Quality of Bread

Four flours of varying strength were selected to be used in this study of the effect of flour strength. These flours were commercially milled from the classes of wheat shown in Table I.

TABLE I

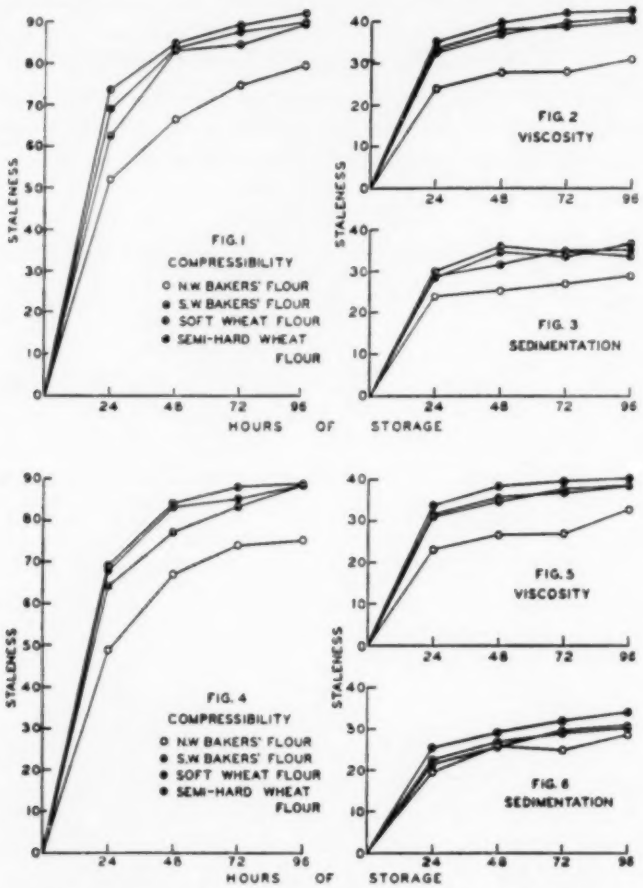
DESCRIPTION OF FLOURS USED IN STUDY OF EFFECT OF BAKING STRENGTH ON BREAD STALING

| Flour number | Flour designation | Wheat from which flour was milled | Crude protein (N×5.7) |
|---|---|---|---|
| | | | % |
| 16699 | Northwestern bakers' flour | Northwestern hard spring wheat | 12.6 |
| 16700 | Southwestern bakers' flour | Southwestern hard red winter wheat | 11.6 |
| 16824 | Soft wheat flour | Southwestern soft red winter wheat | 10.1 |
| 16825 | Semi-hard wheat flour | Nebraska semi-hard winter wheat | 9.6 |

The data recorded in Figures 1 to 3 show the comparative rates of staling of the regular bread and those in Figures 4 to 6 show the staling of soy bread baked from these four flours and stored at 28° C. These staling curves show that the rate of staling of bread is dependent, in part, on the type of flour from which the bread was baked. In general, all three methods of measuring staleness indicated that the loaves baked from the stronger flours did not stale as rapidly nor did they reach as high a degree of staleness as those baked from the weaker, lower protein flours. However, keeping quality of bread does not appear to be strictly a linear function of protein content. Apparently protein characteristics or some other factor or factors inherent in the flour enter into the phenomenon of staling. There was a wider spread in the rates of staling of bread made from the northwestern bakers' flour and the southwestern bakers' flour than there was in the rates of staling of the southwestern bakers' flour and the soft wheat flour, although the difference in protein contents of the first two flours was only 1.0% and that of the second and third flours was 1.5%.

As measured by the compressibility test, the bread made from the 10.1% protein soft wheat flour staled more rapidly than did the bread

made from the 9.6% protein semi-hard wheat flour. This fact also points toward other factors in flour than protein content which account for the differences in keeping quality of bread made from various flours.

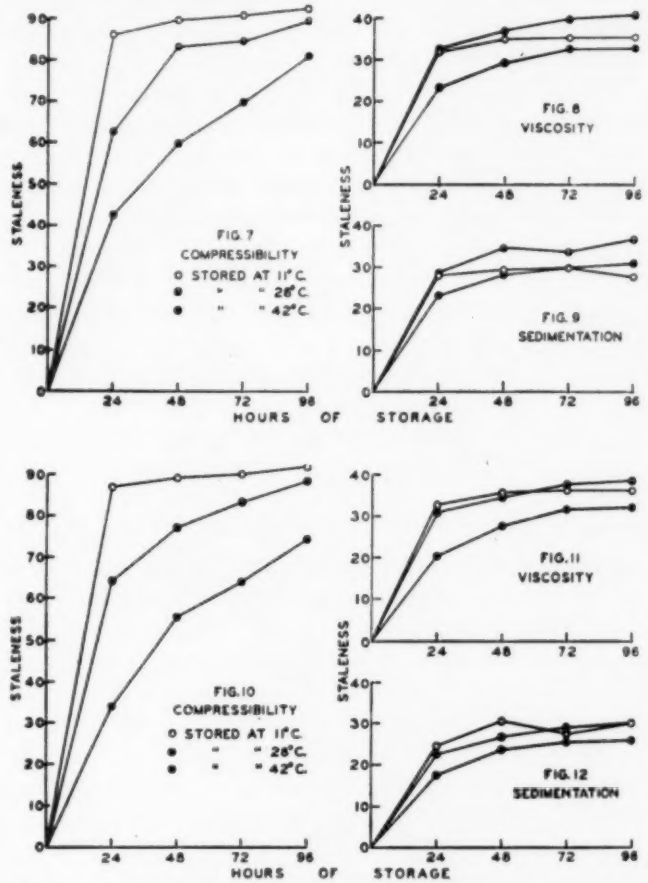



Figures 1 to 6. The effect of flour strength on the staling of bread.

## The Influence of Temperature of Storage on the Rate of Staling of Bread

To study the effect of temperature on staling, bread baked from the southwestern bakers' flour was stored at 11°, 28° and 42° C. Storage at 11° C. is comparable to that often encountered in delivery trucks during the cooler seasons of the year. Temperatures of 28° C. are quite common in stores and homes, especially during the summer months. It was believed that storage at 42° C. would introduce the effect of relatively high temperatures on the rate of staling without causing excessive drying of the bread.

The graphs in Figures 7 to 12 show the extent to which temperature of storage affects the rate at which bread crumb becomes stale. The curves in Figures 7 to 9 represent the staling of regular bread baked from the southwestern bakers' flour and those in Figures 10 to 12 show the staling rates of soy bread. The compressibility test showed that




Figures 7 to 12. The effect of temperature of storage on the staling of bread.

the bread stored at 42° C. did not become as firm as that stored at 28° C. and the latter in turn did not lose compressibility as rapidly as that kept at 11° C. Both the rate of staling and the final degree of staleness as thus measured, even after 4 days' storage, became less as the temperature of storage was increased from 11° to 42° C. Bread stored at 11° C. had very nearly reached its maximum firmness after but 24 hours. On the other hand, bread stored as 42° C. was still quite fresh after 24 hours storage and even after 96 hours was not as firm as

that kept at 11° C. for 24 hours. The staling curves obtained from the data of the compressibility test indicate that as the temperature of storage is raised the staling of bread becomes more nearly a linear function of time.

Although the viscosimetric and sedimentation methods did not afford as consistent and uniform results as the compressibility test, the results obtained by the first two methods indicate that, in general, the lower storage temperature was more conducive to rapid crumb staling. The staling rate was not as rapid, especially during the early storage period, in the bread stored at the higher temperatures. However, at 72 hours' storage the staling rates, as determined by these methods which measure the swelling power of starch, converged closer to the same value. The curves of crumb compressibility did not appear to converge until after 96 hours' storage. In view of the fact that the compressibility method measures a somewhat different change in bread crumb from that measured by the viscosimetric and sedimentation methods, the above observation appears to indicate that the development of crumb hardness tends to lag behind loss of swelling power.

Effects of storage temperature similar to the above were also obtained with bread baked from the spring wheat flour.

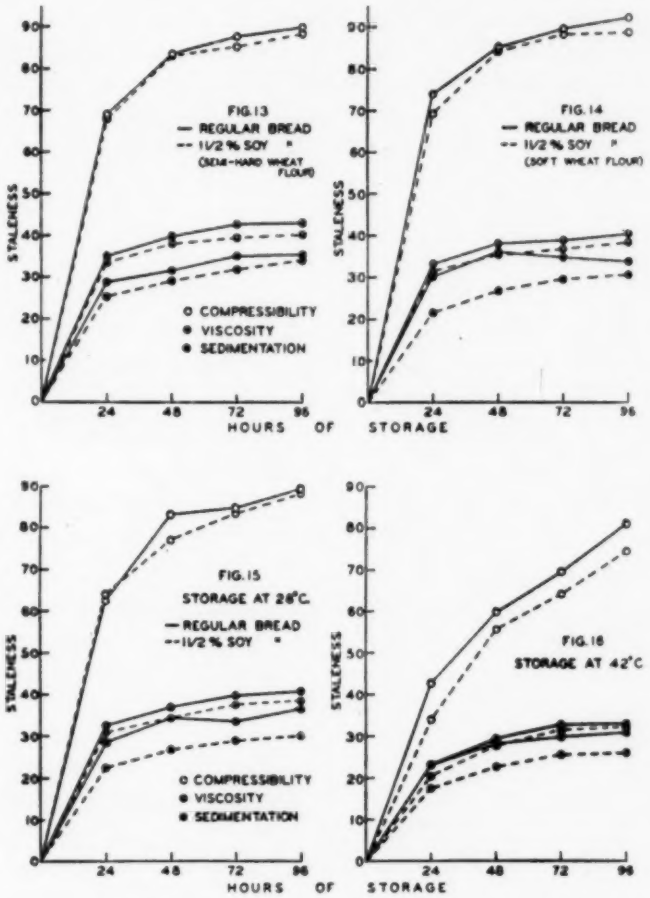
### The Effect of Soy Flour on the Keeping Quality of Bread

To obtain a direct comparison of the rates of staling of the soy bread and the regular bread, the appropriate curves obtained in the foregoing studies are assembled in Figures 13 to 16. The addition of 1½% soy flour to the semi-hard wheat flour and to the soft wheat flour is shown in Figures 13 and 14 to effect some retardation of staling. This bread was stored at 28° C. The rate of staling of the soy bread is represented by the broken-line curves, and it will be observed these curves fall below those of the regular bread, indicating less staling in the soy bread.

Figures 15 and 16 show the effect of soy flour on the staling of bread baked from the southwestern bakers' flour and stored at 28° and 42° C. The difference in the rates of staling of the regular and soy bread was more pronounced when the bread was stored at 42° C. than at 28° C. At the higher temperature of storage the soy bread was fresher 4 to 24 hours after the regular bread had reached any particular stage of staleness.

The use of 1½% soy flour appeared to effect greater retardation in staling in the bread baked from the lower protein flours than that baked from the stronger flours. Although no tests were made it is quite probable that the use of a higher percentage of soy flour would effect a greater retardation of staling than was found with the use of 1½% of

this product. This retardation of staling obtained by the use of soybeam flour is in agreement with the results reported by Straub and Hirsch (1935). Using a mechanical testing device, they found that



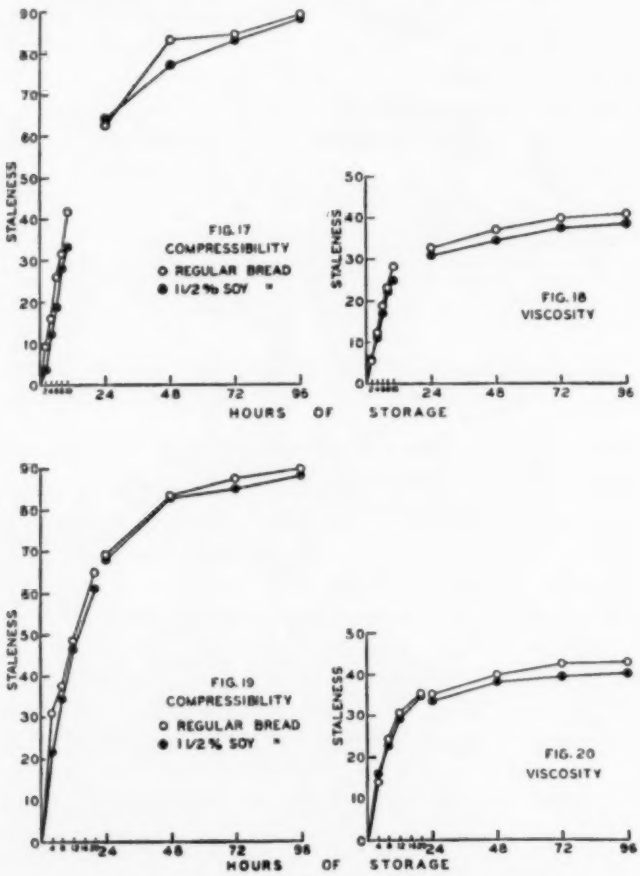

Figures 13 to 16. The effect of soy flour on the rate of staling of bread.

after one to three days' storage soy bread was softer than bread containing no soybean flour.

## The Rate of Staling During Early Storage

Throughout these studies it has been observed that the most rapid staling takes place during the first 24 hours of storage. To study staling during early storage, tests were made on a series of loaves baked from the southwestern bakers' flour. Staling determinations were conducted on the bread one hour after baking and at two-hour intervals

of storage at 28° C. for a period of ten hours. The sedimentation method was not used in this study because it was not sufficiently accurate to detect the small differences which occur during these short intervals of time. In Figures 17 and 18 is shown the very rapid initial rate at which the bread staled. The curves of the rates of staling during the 24 to 96 hour period, as previously shown, were added to these figures to complete the picture.




Figures 17 to 20. The rate of staling during early storage.

The curves in Figures 19 and 20 show the rapid staling of bread baked from the semi-hard wheat flour. The most rapid staling was found to occur during the first 8 to 12 hours out of the oven, after which time the rate rapidly decreased.

### A Comparison of the Methods Employed to Measure Staleness

All three methods gave results which agreed fairly well as to the relative rates of staling of the various types of bread studied. The staleness, after 24 hours' and 96 hours' storage, of the bread baked from the four types of flour as determined by one method was correlated with the staleness of the same bread as determined by each of the other tests. Similar correlations were made of the bread baked from the southwestern bakers' flour and stored at 11°, 28° and 42° C. The coefficients of correlation are shown in Table II.

TABLE II

CORRELATION OF METHODS OF MEASURING STALENESS

| Methods being correlated | Correlation coefficient | |
|---|---|---|
| | At 24 hr. period | At 96 hr. period |
| Bread baked from four types of flour—storage 28° C. | | |
| Compressibility and viscosity | +0.9270 | +0.9045 |
| Compressibility and sedimentation | +0.6146 | +0.7251 |
| Viscosity and sedimentation | +0.6974 | +0.8437 |
| Bread stored at three storage temperatures—southwestern flour | | |
| Compressibility and viscosity | +0.8928 | +0.6986 |
| Compressibility and sedimentation | +0.6973 | +0.3887 |
| Viscosity and sedimentation | +0.8105 | +0.7677 |

The coefficients of correlation of the compressibility and viscosity methods are fairly high; in three of the instances the correlations were found to be about $r = 0.9$, and in the fourth case the correlation was approximately $r = 0.7$. This indicates that these two methods disclosed approximately the same relative rates of staling of the various types of bread which were studied. The correlation of the sedimentation method to the compressibility test ranged from $r = 0.4$ to $r = 0.7$, whereas the correlation of the two methods of measuring crumb swelling was somewhat higher. In the latter case the correlation was $r = 0.7$ to $r = 0.8$.

The viscosimetric and sedimentation methods did not exhibit the wide spread in the rates of staling obtained by the compressibility method. The latter method showed greater effects of flour strength and temperature of storage than did the first two methods. Throughout this study the viscosimetric and compressibility methods gave fairly consistent results which produced reasonably smooth staling curves when calculated as percentage reduction from fresh bread values. Very few irregularities were obtained by these two methods. On the other hand, the sedimentation method often gave results which were quite different from those which were expected. This method is not

sensitive to small differences in the condition of the bread crumb and was of no value in attempting to follow the rate of bread staling during the early storage period. The experimental error of this method was greater than the differences found in the swelling capacity of the bread crumb between successive two-hour periods.

## Summary

Three methods of measuring staleness were employed concurrently to follow the changes in the aging of bread crumb. A measure of the firmness of the crumb was made with an apparatus similar to that described by Platt (1930). This device measures the depression of a plunger into a slice of the bread crumb which is caused by the action of a standard weight on the plunger during a given period of time. Katz's method (1928) of determining swelling power by measuring the volume of sediment obtained from 10 grams of bread crumb was the second test employed. The method of Karacsonyi (1929) was also used to measure the absorbing capacity of the bread crumb. This method involves the measurement of the viscosity of a 10% suspension of finely ground bread crumb.

Flour strength appears to be an important factor in the aging of bread crumb, the rate of staling being a function, though not linear, of the protein content of the flour. Bread baked from the 11.6% protein southwestern winter wheat flour staled much more rapidly at 28° C. than did bread baked from the 12.6% protein northwestern spring wheat flour. Bread baked from the 9.6% protein semi-hard winter and the 10.1% protein soft red winter wheat flours staled more rapidly than did that of the southwestern flour, but this difference was not as great as the difference in the rates of staling of the bread baked from the two stronger flour types, even though the spread in protein content in the former case was greater than in the latter. That protein characteristics may be a factor in the staling of bread is indicated by the fact that, as measured by the compressibility method, the bread baked from the soft wheat flour staled more rapidly than did that baked from the semi-hard wheat flour.

Temperature of storage had a marked effect on the rate at which bread became stale. Bread stored at 11° C. staled very rapidly and was nearly as stale after only 24 hours' storage as it was after 96 hours' storage at the same temperature. Increasing the temperature of storage to 28° or 42° C. greatly retarded the rate of staling, and bread stored at the higher temperatures (28° and 42° C.) did not reach the degree of staleness at the end of the 96-hour period that was obtained at a storage temperature of 11° C. Increased storage temperature appeared to have a greater effect on the compressibility than on the

water-imbibing capacity of the crumb. At 42° C. the percentage decrease in compressibility was nearly a straight line function of time.

Soybean flour added to the bread dough tends to retard the staling of the baked bread. The use of 1½% fat-free soy flour was found to reduce both the rate and degree of staling of bread baked with various types of wheat flour. This retardation was more evident in the bread stored at the higher temperatures (28° and 42° C.); the soy bread remained fresh 4 to 24 hours longer at 42° C. than did the regular bread.

During the first 8 to 12 hours out of the oven, the bread was found to undergo the most rapid changes in crumb characteristics. After such intervals the decrease in compressibility and the decrease in water-absorbing capacity of the crumb were nearly linear functions of time. Following this initial period of rapid staling, the rate decreased rapidly.

Compressibility and viscosity measurements of staleness were more consistent and uniform than the data obtained by the sedimentation method. The effects of flour strength and temperature of storage were more pronounced in the compressibility measurements than in the data obtained by the other two methods. The sedimentation test is not sensitive to minor changes in the condition of the bread and is subject to error due to faulty settling of the crumb.

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# A COMPARISON OF TWO SIZES OF BAKING PANS ON 100-GRAM FLOUR DOUGHS PRODUCING LARGE LOAF VOLUMES[1]

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In evaluating the baking-strength qualities of small samples of wheat grown at the various experimental stations of the Dominion Experimental Farms System, the Cereal Division for some years has been using as the main baking formula the American Association of Cereal Chemists' standard baking formula with the additions referred to by Larmour, Geddes, and Whiteside (1933) of .001 g. $KBrO_3$, .1 g. $NH_4H_2PO_4$ and .3 g. of a high diastatic malt extract (250° L.). This formula, when applied to high-protein flours milled from wheats grown in certain areas of the prairie provinces, has been giving loaves of exceptionally large volume; it is not uncommon for some of them to exceed 1,000 c.c. The low-form baking tin of the dimensions specified by Geddes (1934) has not been entirely satisfactory since the development portion of the loaves, classified by Blish (1928) as being the portion of the loaf above the pan, was much in excess of the base of the loaves, that portion within the pan, and the occurrence of large holes under the crusts at the top of the loaves was not unusual. Consequently, a number of baking pans of different sizes and shapes were employed to find out if more desirable test loaves might be produced by altering the size of the pan.

## Experimental

After some preliminary trials in which a number of test bakes were made with the A. A. C. C. high and low pans and two other types slightly larger than the Pioneer pan described by Davis, Leatherock, and Putnam (1936), the pan hereafter described as the "large" pan was chosen for comparison with the A. A. C. C. low-form tin. The two pans have the following dimensions:

| | A. A. C. C. low-form pan Cm. | Large pan Cm. |
|---|---|---|
| Top width | 7.0 | 9.5 |
| Bottom width | 5.5 | 7.5 |
| Top length | 11.5 | 12.8 |
| Bottom length | 9.5 | 10.4 |
| Height | 5.0 | 6.5 |

[1] Contribution No. 107 of the Cereal Division, Dominion Experimental Farm, Ottawa, Canada.

The approximate capacities are 350 c.c. and 650 c.c. for the A. A. C. C. low-form pan and the large pan respectively. The pans were both constructed from 2 xx tin.

Five flours, 2 commercially milled and 3 experimentally milled, were employed in the baking test using the above mentioned formula. The flour designations and protein contents are given in Table I.

TABLE I

PROTEIN CONTENT OF 5 FLOURS USED IN THE EXPERIMENT

| Sample number | Flour protein (13.5% moisture basis) |
|---|---|
| | % |
| 1 | 13.2 |
| 2 | 13.8 |
| 3 | 12.9 |
| 4 | 15.1 |
| 5 | 17.0 |

Samples No. 1 and No. 2 were commercial bread flours, the former a short patent and the latter a long patent flour. Samples 3, 4 and 5 were straight-grade flours milled in an Allis-Chalmers experimental flour mill. These five flours were selected for the range in loaf volumes which they afforded even though the baking formula and procedure were possibly not well suited for samples 1 and 2 since they were already chemically improved. Loaves were baked from these flours in both types of pans on each of 14 consecutive days, and in Table II the mean loaf volumes with their standard errors for the 14 bakes and coefficients of variability are given for each flour employing each of the two pans.

TABLE II

STATISTICAL DATA FOR LOAF VOLUMES OBTAINED WITH THE TWO PANS

| Sample number | Mean loaf volume in c.c. | | Coefficient of variability | |
|---|---|---|---|---|
| | A. A. C. C. pan | Large pan | A. A. C. C. pan | Large pan |
| 1 | 587 ± 8.55 | 608 ± 12.03 | 5.5 | 7.4 |
| 2 | 735 ± 10.69 | 720 ± 9.35 | 5.4 | 4.9 |
| 3 | 859 ± 8.55 | 861 ± 8.55 | 3.8 | 3.7 |
| 4 | 938 ± 11.76 | 950 ± 5.35 | 4.7 | 2.1 |
| 5 | 1094 ± 9.35 | 1116 ± 8.82 | 3.2 | 3.0 |

It will be noted in Table II that no significant differences were obtained for the mean volumes of the loaves baked in the large pan over those baked in the A. A. C. C. low-form pan and that no improvement in replicability was shown for the former pan as indicated by

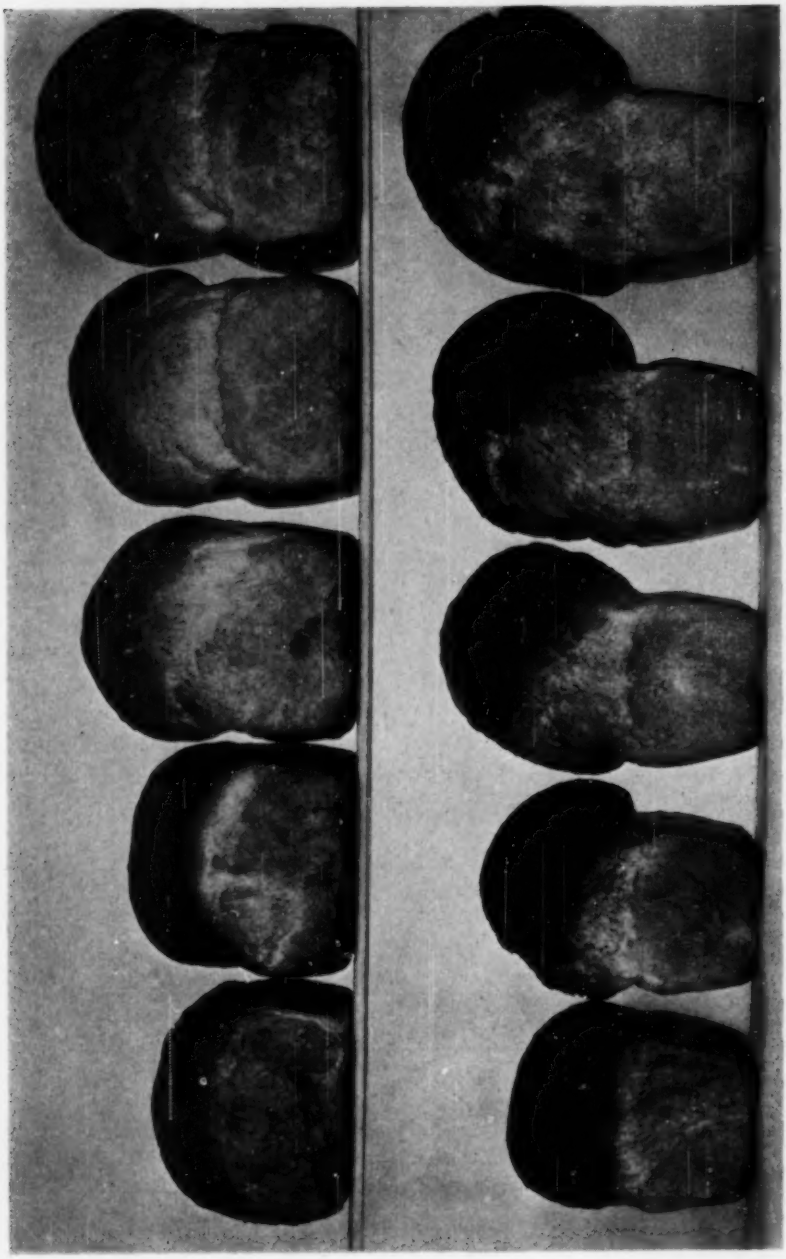

Figure 1. From left to right uncut loaves from flours Nos. 1 to 5. Top row baked in large pan; bottom row baked in A. A. C. C. low pan.

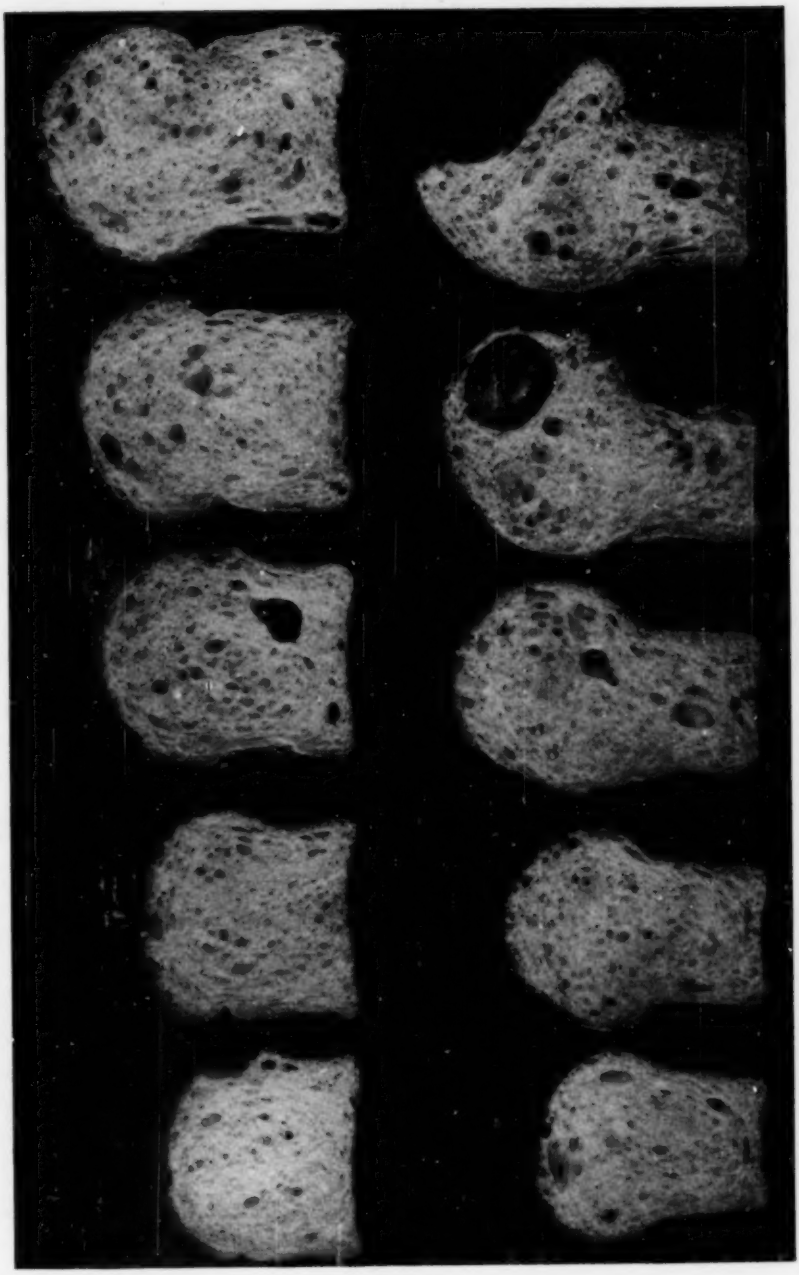

Figure 2. Cut loaves as in Figure 1.

the calculated standard errors or the coefficients of variability. This was surprising in view of the fact that the large pan did not produce large holes in the development part of the loaf although these occurred fairly frequently in the loaves baked from flours 3, 4 and 5 employing the A. A. C. C. low-form pan.

In Figures 1 and 2 the external and cut surfaces of the loaves for the 5 flours numbering from left to right, baked on one of the days, are represented. The superiority of the large pan for producing more uniformly shaped loaves, especially for flours 3, 4 and 5, is evident. Moreover, the tendency for the A. A. C. C. low-form pan to produce large holes in the development part of the loaf is shown for the loaves baked from flours 4 and 5. This tendency, it is believed, is due in part to the lack of protection given to the upper part of the proved dough by the smaller pan when doughs of very strong flours are first placed in the oven, as this fault has been corrected when the larger pan was used. The crumb structure of the loaves baked in the larger pan is inclined to be a little more open, but more uniform throughout, than when the smaller pan was used. The loaves such as those produced from flours 3, 4 and 5, baked in the smaller pan, produced crumb structures which are not uniform as the upper or development part of the loaf is more open than the lower or base part. It is, therefore, clear that the relation of the dough to the top of the pan at oven time influences both the internal as well as the external characteristics.

## Summary

Two baking pans, the A. A. C. C. low-form tin and a larger pan, have been compared employing 5 flours of varying protein contents. For very strong flours the larger pan produced loaves of better external appearance, eliminated the tendency of large holes occurring under the crust, and gave more uniform crumb structures, although slightly more open, than did the smaller pan. The change in pan size did not alter, appreciably, the loaf volumes nor did the larger pan appear to improve the replicability of bakes on different days. As would be expected, the A. A. C. C. low-form pan gave more desirable appearing loaves with volumes under 700 c.c.

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# REPORT OF THE A. A. C. C. METHODS COMMITTEE

R. M. SANDSTEDT, *Chairman*

Department of Agricultural Chemistry, University of Nebraska, Lincoln, Nebraska

(Read at the Annual Meeting, May 1937)

## General Report

The Methods Committee during the past year has developed and studied the following methods or adaptations of methods:

A method for sucrose in flour, a short gassing power method, a 15-minute moisture method for flour, and an adaptation of the ferricyanide method for high diastatic flours and for sucrose. The subcommittee on viscosity has continued its studies. An attempt has been made to compile and present to the Association various short-cuts and improvements in methods, and laboratory devices or "gadgets" which the members of the Association have developed and find useful but which are ordinarily not available to the general membership of the Association.

## Recommendations as to Policy

It has been the policy of the Association to take no part in giving direction to the Methods Committee work. A new committee is chosen each year, each member of which selects his own problem for work. This is a "hand-to-mouth" policy which provides no definite objective. Accordingly it is recommended that the policy of the Association toward the Methods Committee be so changed that direction may be given to the committee work and the committee given definite objectives. This may be accomplished by selecting the methods to be investigated in advance of the appointment of the membership of the committee. The methods selected should be those for which the Association feels a need and the members of the committee should be chosen because of their special qualifications to work on these particular methods. This change in policy would place the members of the Methods Committee on a basis similar to that of the associate referees of the Association of Official Agricultural Chemists.

It is further recommended that the collaborative facilities of the Sections of the Association be used, with limitations, for further development of those methods recommended by the Methods Committee; that is, that in so far as the sentiment of the various Sections is favorable, the collaborative checking of the Sections be extended

to include those improved methods in which the particular Section is interested. This would greatly facilitate the improvement of methods by proving or disproving their value more speedily. It would also put desirable changes into general use more rapidly. It is also highly desirable that the other committees make use of certain recommended procedures in their work, *e.g.*, those using viscosity determinations should make use of the technique recommended by the subcommittee on viscosity.

## Recommendations on Methods

It is recommended:

That the methods developed this year be further studied collaboratively.

That the collection and disemination of knowledge concerning "short-cuts and gadgets" be continued.

That the following recommendations held over from last year's methods committee be given consideration.

"That more extended research be done relative to the evaluation of shortening, milk, malt, yeast, yeast foods and other ingredients which are used in breadmaking.

"That the subject of laboratory experimental milling be further studied and an effort made to approach more uniform conditions in laboratory milling procedures.

"That methods for the measurement of the proteolytic activity of flour be investigated."

# ERRORS INVOLVED IN THE MEASUREMENT OF GAS PRODUCTION BY THE FERMENTOGRAPH

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(Received for publication August 2, 1937)

## Introduction

The Fermentograph is one of the instruments in use for the determination of gas production in doughs. This instrument employs the law of buoyancy in recording the volume of gas evolved in a dough; a body immersed in a liquid is buoyed up by a force equal to the weight of the displaced liquid in accordance with Archimedes' principle.

The Fermentograph has the advantage of automatic graphic recording of the amount of gas produced. However, various factors interfere with the true recording. The machine is of great use for routine tests but for high precision work leaves much to be desired.

The Brabender Company [1] lists the following factors to be considered in the calibration of the instrument:

1. The weight of carbon dioxide in the balloon.
2. The change of the specific weight of water at 30° C. and 4° C.
3. The compression of the carbon dioxide in the rubber balloon and in the dough medium.
4. The pressure of the water column on the rubber balloon and the consequent change in volume of gas.

In addition to the factors enumerated above, our experience with the Fermentograph has shown that other important causes of error must be taken into account. These are:

1. Diffusion. Carbon dioxide is a water soluble gas, and rubber is a permeable membrane allowing the gas to diffuse and dissolve in the water bath.
2. Stretching of the rubber balloon.
3. Punching of the balloon.
4. Mechanical defect in the balance head.

First of all, it was found that when identical doughs were placed in all four units, the checks were not as close as was desired. The scale

[1] Private communication.

heads were then checked one against the other and mechanically adjusted to give uniform readings from unit to unit. Another experiment was conducted in which doughs were prepared with identical formulas, but the quantity of dough placed in each balloon was varied. One would expect to obtain double the amount of gas if one had double the amount of dough, but this was not the case. From experiments such as these, it was concluded that an attempt should be made to evaluate the individual errors and measure, as far as could be done, the quantitative effect of each error.

## Discussion

*Weight of carbon dioxide in the rubber balloon.* The maximum amount of carbon dioxide present at any one time can not be more than 1000 c.c. If 22.4 l. of carbon dioxide weigh 44 g., one liter would weigh $\frac{44}{22.4}$. This would be less than 2 g. and since it would record about 2 c.c. it is a negligible error. Carbon dioxide, of course, is not the only gas present since alcohol, water vapor and some volatile organic compounds comprise a portion of the gas. These latter, however, are not present in any significant quantity.

*The specific weight of water.* The density of water at 30° C. is 0.995646 or 1000 c.c. are buoyed by a force equal to 995.6 g. and not 1000 g.

*Compression of carbon dioxide in the rubber balloon and in the dough.* No attempt was made to evaluate this factor since it was not known just what was meant.

*Pressure of the water column on the balloon and the consequent change in volume due to this additional pressure.* The balloon is immersed in the water so that its top is about 2 inches from the surface of the bath and its bottom about 8 inches from the surface. The rise of the balloon in traversing the entire range of the 1000 c.c. on the graph is approximately three-quarters of an inch. Therefore, after the development of 1000 c.c. of gas the balloon is closer to the surface by about three-quarters of an inch than it was at zero cubic centimeters. For the sake of a rough computation, let five inches be taken as the average length of the column of water over the balloon. The balloon is then under a pressure of the atmosphere plus 5 inches of water. Five inches of water are equivalent to approximately 9.5 mm. of mercury and thus the pressure on the balloon is 760 plus 9.5. Therefore, 1000 c.c. immersed under a column of 5 inches of water become 987. The variation in pressure due to the rise of the balloon can not affect the volume by even one cubic centimeter.

## Experimental

### Diffusion

The error due to diffusion is large and the measurement of it was attempted in the following manner. Carbon dioxide gas developed during the fermentation in a dough-filled balloon was used to inflate an empty balloon. The balloon filled with carbon dioxide gas alone was placed in its holder and suspended in the water bath. The loss of gas was noted by the fall of the pointer on the graph. The loss varied with the amount of gas in the balloon; about 30 c.c. diffused through the balloon in an hour when it was inflated to approximately 150 c.c. of gas and 60 c.c. diffused when the gas volume in the balloon was 1000 c.c. In order to be sure that the loss was due to the solubility of carbon dioxide and could not be attributed to leaks in the balloon, air was substituted for the carbon dioxide. No loss of air was observed.

It was hoped that a heavier rubber might stop the diffusion or reduce it to a negligible minimum. With this in view, a home-made balloon was constructed from automobile inner tubes and tire patch. The diffusion was not stopped but was considerably lessened; it amounted to about 16 c.c. per hour, constant more or less over the entire range. Experiments on the inner tube balloon showed that diffusion, even when it is diminished by use of the heavier rubber, remains quite an error. Identical doughs, except for size, were fermented in the heavy rubber balloon. A reading of 551 c.c. was recorded for a 100-g. dough, and 868 c.c. for a 150-g. dough. Three halves of 551 is 825 and this figure falls far below the figure actually obtained for the 150-g. dough. However, if a correction is made for diffusion, more reasonable results are obtained. The doughs were fermented for a four-hour interval and according to a diffusion factor already determined, the doughs lost 64 c.c. by diffusion during this time interval. When this amount is added to each figure, 615 c.c. are obtained for the 100-g. dough and 932 c.c. for the 150-g. dough. If 615 is multiplied by 3/2 a figure of 923 is found. This is only 9 c.c. from the figure of 932 for the 150-g. dough, a fair experimental error.

A further proof that the diffusion is due to the solubility of carbon dioxide in the water was shown by experiments in which the dough was fermented in lubricating oil baths and saturated salt solutions only replacing them in the regular water bath at the hourly intervals to register the volumes on the graph. Between 4 and 5% more gas was obtained by fermenting in such media than in water.

There are two methods by which diffusion could be eliminated, one to make the balloon out of a material that is impermeable to the carbon dioxide and the other to substitute some liquid, in which carbon dioxide

is insoluble, for the water in the constant temperature bath. The difficulty of substituting some material for the rubber is in the manufacture of the article. Duprene, for example, is known to be less porous to gasses than ordinary rubber, but one can not obtain Duprene balloons of the size and shape needed. The ideal liquid for the bath should have these properties: It should not dissolve carbon dioxide, it should have a density of one, should not harm rubber, nor be prohibitive in price.

### Stretching of the Rubber

An error of some magnitude can be introduced by the stretching of the rubber balloon. The capacity of the balloon unstretched is about 1100 c.c. If a dough weighing 400 g. is placed in the balloon it can be seen that in order that 1,000 c.c. be recorded, the balloon must be stretched. Experiments showed that an expanded or stretched balloon recorded 20 c.c. less than an unstretched balloon for a 400 c.c. volume.

### Punching of the Balloons

It is recommended by the manufacturers to punch down the dough at the end of each hour and expel the gas from the balloon. This is necessary if a sizable dough is used in order to make room for the gas developed during the later hours of fermentation. This can be circumvented by using a smaller dough so that the total gas developed over the entire fermentation does not exceed 1000 c.c. Then the balloon need not be punched but in this method other errors such as diffusion are great relative to the dough size.

In punching the balloon, between 1½ and 2 minutes are required. This is a 2-minute loss between every hour of dough fermentation and in 6 hours amounts to 10 minutes. Though the loss in time can be kept quite constant, the loss in unrecorded gas volume is an uncontrollable figure. At the height of fermentation a dough may be producing 16 c.c. per minute and in the later stages only 3 or 4 c.c. With this variation in the rate of gassing power, the gas loss can not be kept constant or easily computed when different flours and different formulas are used.

### Mechanical Defect in the Balance Head

In order to determine how the machine recorded with known amounts of gas, the following set-up was used. A gas burette measured successive increments of gas placed in the balloons and the Fermentograph recording was noted. The gas used was air. The temperature regulator on the bath was turned off to eliminate a temperature error of measuring the gas at room temperature in the gas burette and at 30° C. in the water bath.

For an interval from 0 to 500 c.c. the Fermentograph averaged 79 c c. for each 100 c.c. of air, and from 500 to 1100 c.c. it averaged 90 c.c. It can be seen that the recording is not uniform from one end of the graph to the other. The lower readings on the Fermentograph can be accounted for by the fact that Brabender states the machine has been calibrated to reduce the readings to standard conditions of temperature and pressure. Doing this we obtain:

Temperature.....25° C.
Barometer.....737 mm.

$$\frac{1000}{1} \times \frac{737}{760} \times \frac{273}{273 + 25} = 888.$$

For 1000 c.c. of air, the machine records 870 c.c., an error of 18 c.c. Unless each machine is adjusted individually to compensate for altitude differences under which each will be operating, the mechanical computation for reduction to standard conditions will never be correct.

**Summary**

An evaluation of the errors involved in the measurement of gassing power by the Fermentograph is given. From the results of our work all the errors, except diffusion, can be overcome successfully and compensated for, if the machine is calibrated with known amounts of gas. The diffusion factor, however, is too large an error to be disregarded, and in order to make the Fermentograph entirely reliable the diffusion factor should be taken into consideration.

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## THE MARCH OF EXPANSION AND TEMPERATURE IN BAKING BREAD[1]

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(Received for publication March 8, 1938)

The march of temperature in bread dough during ordinary oven baking has been the subject of several studies reported in the literature. Rate of temperature increase has been demonstrated to be a function of the size of the dough mass, as well as of the temperature of the oven. It has appeared, generally, that the temperature finally attained by the interior of the loaf is in the range of the temperature of boiling water at the barometric pressure where the baking is done. In none of these earlier studies, as far as we are aware, has the march

[1] Paper No. 1596, Journal Series, Minnesota Agricultural Experiment Station.

of temperature been related with the relative expansion of the dough. The studies here reported were accordingly directed toward that end.

Our first investigations were undertaken with pan bread, that is, with dough baked in an iron pan. The relative dimensions of the pan are indicated by the cross section of the baked loaves shown at the top of Figure 1. Near the bottom of the pan the slices were 10 cm. across, increasing at the maximum to 12 cm. as shown in the diagram. Vertically the slice approached 15 cm. in height. The quantity of dough was 500 g. in each instance.

At the time of molding or forming the doughs before placing them in the pans, thermocouples were inserted into the interior of the mass. The position of these thermocouples at the conclusion of baking was noted and is recorded by small open circles in Figures 1 and 2. These thermocouples were copper-constantan, with two hot junctions in series in the dough and the cold junction in melting ice (0° C.) in a thermos bottle. The potential of this multiple thermocouple was measured by means of a pyrovolter, and the values in E.M.F. converted into terms of ° C. after careful calibration of the thermocouples. The doughs were "proofed" or allowed to ferment in the pan at 30° C. in the customary manner, and then placed in an electrically heated oven maintained as closely as possible at a temperature of 200° C.

Under these conditions, in an oven in which the water-vapor is trapped so that the oven atmosphere is relatively humid, a dough made with strong flour, properly fermented, will expand or "spring" considerably. Thus a typical dough made from strong, high-protein flour and weighing 500 g. had a displacement of 1670 c.c. when placed in the oven, and of 3040 c.c. when baked, or an increase in volume of 1370 c.c., equivalent to 82%.

It was impossible to measure conveniently the actual displacement of the dough at intervals during the baking process. In lieu of this, a device was arranged in the oven by means of which the relative height of the dough could be observed at any time. The changing height is approximately proportional to the increase in volume, at least to the extent of enabling us to estimate the relative increase in displacement during baking. Such observations were made in the instance of three series of pan loaves, concurrently with the measurements of temperature by means of the thermocouple, and are recorded in Figure 1.

In the instance of loaves No. 1 and No. 2 where the thermocouples were about half way between the surface and the center of the loaf, the initial rate of temperature increase was somewhat greater than in the center of loaf No. 3. The vertical expansion of these same loaves,

recorded by the curves marked "E," was most rapid during the first 5 or 6 minutes of baking when the interior of these loaves was in the range of 40±° C. Expansion was practically concluded at the end of the 10th minute when the interior of the same loaves was in the range of 50° to 60° C. Thus the actual increase in dough temperature was only 20° to 30° when expansion ceased, although the dough still increased in temperature 40° to 50° more before the baking process was concluded.

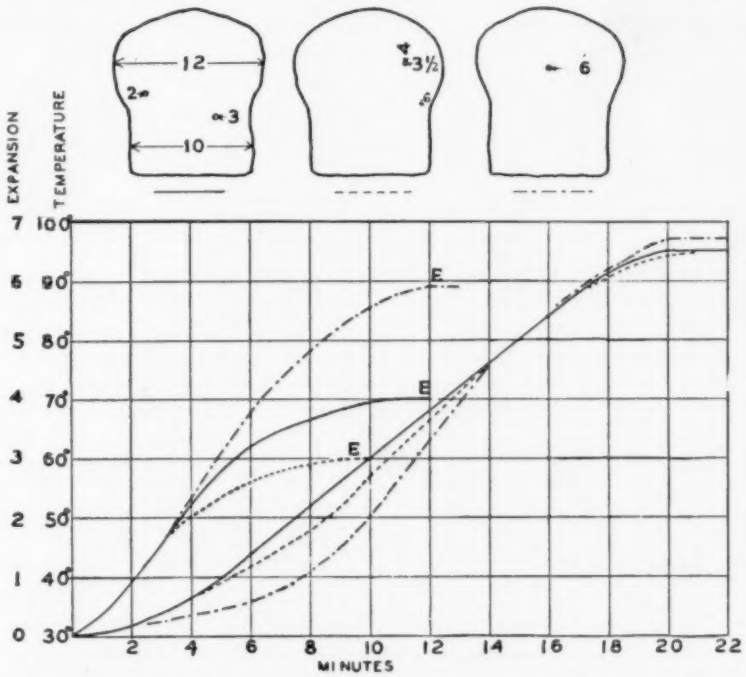


Figure 1. Observations on pan bread during baking. Curves E record expansion in centimeters measured in vertical height. Curves terminating at the right record the changes in temperature in the interior of the doughs during baking at 200° C., temperatures being measured at the positions shown in the sections illustrated alone and with the distance in centimeters from the outer crust being shown in each case.

Another series of experiments was then conducted with "hearth bread," that is, loaves baked on the hearth of the oven and not enclosed in a pan. Measurements in this instance are not as simple or satisfactory as with pan bread where the expansion is directed vertically to a considerable degree. The observations, as recorded in Figure 2, are of the same general nature as those made with pan bread, although expansion continued for about 2 to 4 minutes longer and the rate of temperature increase was somewhat slower. The cessation of expansion came at about the same temperature, namely 60±° C.

These observations lead to some speculation respecting the physical and biochemical bases for dough expansion in baking. The following factors might be proposed:

1. Thermal expansion of gases held as such in the vesicles of the dough.

2. Distillation of $CO_2$ from the dough solution and its expansion with the elevation of temperature.

3. Distillation and expansion of water vapor.

4. Accelerated diastatic activity and fermentation rate in the dough as the temperature is elevated into the range of 55° C., above

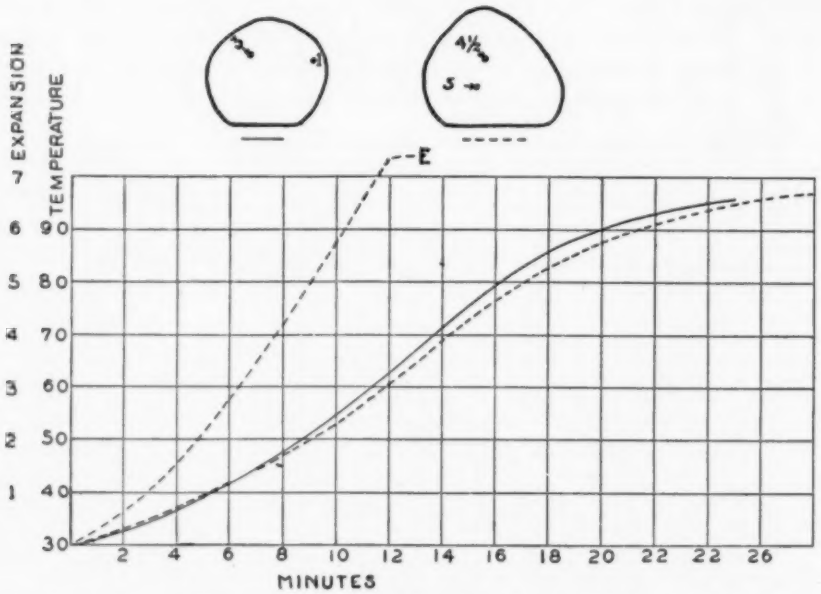


Figure 2. Observations on hearth bread during baking (see legend under Figure 1 for details).

which temperature the rate of such enzymic processes may be progressively reduced.

5. Changes in dough mobility or consistency which may facilitate expansion as induced by the factors outlined in 1 to 4.

It is not easy to measure accurately the individual contributions made in each of these instances. Approximate calculations involved in 1 and 2 would suggest that they were scarcely adequate to account for all the expansion of dough in baking. It must be conceded, however, that we do not know how much $CO_2$ is dissolved in the dough solution. Accelerated enzymic activity cannot be overlooked as a contributing factor when dough is heated progressively in baking.

Using the farinograph as a means for measuring the influence of temperature upon dough consistency, a series of observations was made at temperatures from 30° C. to 80° C. All these doughs were mixed with the same ratio of flour to water and consequently had the same mobility at 30° C. Two series of doughs were prepared with (1) a low-diastatic flour and (2) the same flour to which 0.37% of

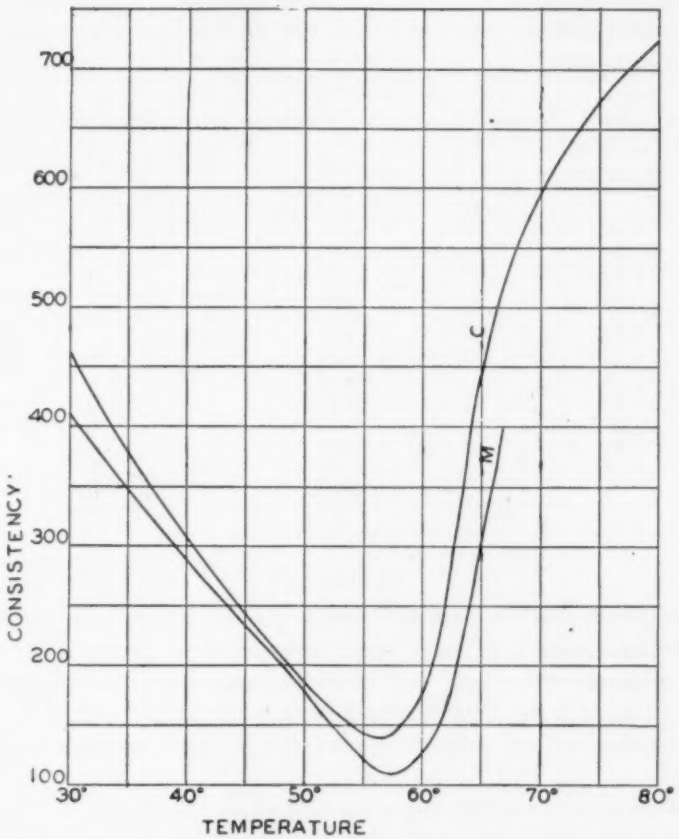


Figure 3. Relative consistency of doughs, measured with the farinograph, as a function of temperature. Curve marked C represents a simple flour-water dough, while curve marked M represents the same flour to which 0.37% of wheat malt had been added.

wheat malt had been added. The changing consistencies of the doughs in farinograph units, as a function of temperature, are recorded in Table I and graphed in Figure 3. It will be observed that the dough containing malt was more mobile than the reference or control dough. In general, the doughs became more mobile, *i.e.*, registered a lower farinograph unitage, as the temperature was increased to 57° C. With further elevation of temperature, the mobility decreased.

It is conceivable that the increased mobility of dough may facilitate expansion in baking. Certainly it is notable that expansion continued through that temperature range in which the dough was more mobile than when first placed in the oven.

As heating continues during the operations of baking, the dough becomes decidedly less mobile, notably between 62° and 67° C., and ultimately assumes a more or less rigid state in consequence of the heat denaturation or coagulation of the proteins, and the gelatinization of the starch.

TABLE I

RELATIVE MOBILITY IN FARINOGRAPH UNITS AT DIFFERENT TEMPERATURES

| Temperature °C. | Malt-free flour dough | Flour +.37% wheat malt in dough |
|---|---|---|
| 30 | 460 | 410 |
| 34 | 410 | 360 |
| 35 | 360 | 330 |
| 38 | 340 | — |
| 40 | 310 | 300 |
| 42 | 290 | — |
| 45½ | 250 | 230 |
| 47 | 215 | — |
| 48 | 210 | 200 |
| 51½ | 170 | 160 |
| 57 | 140 | 110 |
| 62 | 220 | 160 |
| 67 | 570 | 400 |
| 80 | 720 | — |

## Summary

Oven expansion or "spring" involving the increase in volume or cubical displacement of pan bread amounted to about 80% of the dough displacement when first placed in the oven. This expansion was rapid during the first 10 minutes of baking, and ceased at about the time the median temperature of the dough passed through 50° to 60° C.

Median dough temperatures continued to rise during the next 10 or 12 minutes, however, and ultimately reached the temperature of boiling water after the 20th or 22nd minute.

Hearth bread behaved similarly except that the oven expansion or spring continued about 2 to 4 minutes longer, and the rate of temperature increase was a little slower.

Oven expansion is attributed to a combination of thermal expansion of gases or vapors present in or distilled from the dough, acceleration of enzymic phenomena in the early stages of baking which results in increased gas production, and all of these possibilities facilitated by increased dough mobility up to the time the dough becomes rigid in consequence of protein denaturation and gelatinization of the starch.

## BOOK REVIEWS

**Basic German for Science Students.** Third Edition. By M. L. Barker. Published by the Chemical Publishing Co. of N. Y., Inc., New York, N. Y. 186 pages. 1937. Price $2.50.

Dr. Barker's fundamental idea is to have the student learn, through the medium of his book, a reading knowledge of German adequate to give in English the gist of the German passage set in his Final B.Sc. paper (Great Britain) and to understand German articles and periodicals dealing with his special subject or research work. The demand for a third edition is evidence that its objective is being met. In this new edition is incorporated more of the recent passages for translation in the B.Sc. examinations of the Universities of London and Edinburgh.

The Introduction and Part I provide the student with the material necessary for acquiring a vocabulary of approximately 650 words of the first thousand basic words, *i.e.*, those words which are actually of most frequent occurrence in the writings of the German people.

The discussion of the German grammar has been reduced to the absolute minimum necessary for acquiring a reading knowledge of German.

The chief aim of Part II is to provide the student with a wide range of short scientific extracts, including chemistry, zoology, botany, physics, mathematics and medicine. In addition, the study of the first thousand basic German words and their most common derivatives is continued. Taking Parts I and II together, the total number of basic German words (including common derivatives) contained in the passages is approximately 1400.

Dr. Barker has presented in his book a satisfactory means for introducing the student into a knowledge of scientific German, but emphasis should be placed on the statement found in the introduction to the first edition, namely, that the book "will gain vitality and effectiveness in the hands of a teacher."

F. L. DUNLAP

**The Determination of the Amino Acids.** By Richard J. Block. Published by Burgess Publishing Co., Minneapolis, Minnesota. 85 pages. Price $2.00.

The author gives all essential details of the selected methods for the determination of arginine, histidine, lysine, tyrosine, tryptophane, dihydroxy phenylalanine, thyroxine, proline, hydroxy proline, cystine, cysteine, methionine, glutamic acid, hydroxy-glutamic acid, aspartic acid, alanine, glycine, leucine, phenylalanine, and serine. In several instances the amino acids are considered in groups having related properties. Several methods are given for certain amino acid determinations when useful alternative procedures are available. The author has appended many useful footnotes to each chapter, including detailed descriptions of methods for the preparation of reagents, precautions to be observed at various stages in the determinations and other pertinent suggestions to smooth the path of the less experienced worker who is venturing into this field for the first time. In fact, Dr. Block gives such straightforward procedures as to deprive protein studies of much of their terrors, and the only caution the reviewer would sound is that possibly certain of these procedures are not quite as easy, nor as precise as might appear from reading the manual.

The final chapter of the book (Chapter XI) is comprised of outlines or "flow sheets" for the separation of amino acid mixtures including I. Fischer method, II. Cherbuliez method, III. Dakin method, IV. Brazier method, and V. Method of Przylecki and Kasprzyk. The reviewer suggests that this section of the book permits of considerable useful expansion to cover more details of these fractionation techniques, and precautions which must be observed at various stages.

Dr. Block has rendered a real service to workers in this field in organizing such a convenient manual. It is to be hoped that he will continue with this task, and will later add to the manuscript as new methods become available.

C. H. BAILEY

# ANNUAL REPORT OF TREASURER

OSCAR SKOVHOLT

January 1, 1938

The Treasurer's Annual Report is presented in the same simple though complete form as established by previous incumbents. A continuing gain in membership is shown, although the increase is smaller than in the previous year. The surplus from the year's operations is greater. Only a few points will be made by way of comment:

In the statement of membership, the listing of "Members lost by death" has been substituted for "Members deceased during the year." The reason is that the death of a member that occurs during a year will not be reflected as a loss in membership until the following year if the current dues have been paid.

The 1937 Convention in Minneapolis was so conducted that a substantial surplus was unintentionally accumulated which was donated to the Association. The Executive Committee voted to place enough of this money into the Convention Reserve Fund to bring this to an even $1,000.00 in amount. In the opinion of the Committee, this may be a sufficient reserve in this fund. The remainder of the surplus was expended in increasing the size of the 1937 volume of Cereal Chemistry.

The reprinting of Volume I, No. 1 of Cereal Chemistry was an extraordinary item of expense that was paid out of the year's receipts with a small surplus remaining to increase the reserve of the Cereal Chemistry fund.

The increase in surplus of general association funds was larger than usual due to the Convention surplus and the lack of any extraordinary expense except that of the Kansas City Exhibit.

"Cereal Laboratory Methods" nearly maintained the rate of sale of the previous year. At the close of the year, a total of 81 bound volumes was on hand with a further supply of 137 volumes at the printer's in unbound form. The rate of sale in recent months indicates that the supply may be exhausted by about the end of 1940, although unforeseen developments may change this date in either direction.

In the statement of the Distribution of Net Assets, entries have been made of *Interest Earned During 1936* that were not shown on last year's statement.

Savings accounts are distributed more widely than advisable for convenience even when due consideration is given to safety. The assets in Building and Loan Stock in Kansas City are carried at the reduced rate as authorized in 1936. Some reorganization is being completed that will result in a transfer of these assets to another concern, but their actual value cannot yet be determined.

## DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1937

| | Total | Active | Corp. | Hon. |
|---|---|---|---|---|
| Membership December 31, 1936 | 539 | 490 | 47 | 2 |
| New members added during 1937 | 43 | 41 | 2 | — |
| Members reinstated during 1937 | 12 | 12 | — | — |
| Members resigned and suspended for non-payment of dues during 1937 | *29* | *27* | *2* | — |
| Members lost by death | *1* | *1* | — | — |
| Members in good standing as of December 31, 1937 | 564 | 515 | 47 | 2 |
| Net increase in membership during 1937 | 25 | 25 | — | — |

## PROFIT AND LOSS STATEMENT

January 1 to December 31, 1937

| | |
|---|---|
| RECEIPTS | |
| Cereal Chemistry | |
| Membership Dues | |
| Active | $1,809.50 |
| Corporation | 470.00 |

| | | | |
|---|---|---|---|
| Subscriptions, reprints, back issues and advertising | $6,775.88 | | |
| 1937 Accounts Receivable | | | |
| Less: 1936 Income received in 1937 | *1,166.96* | | |
| Net 1937 | | 5,608.92 | |
| Interest on Invested Funds | | 56.81 | |
| Surplus from Minneapolis Convention | | 287.34 | |
| Total Net Receipts 1937 | | | $8,232.57 |
| Association | | | |
| Membership Dues | | 1,802.50 | |
| Application Fees | | 123.00 | |
| Interest on Invested Funds | | 64.66 | |
| Surplus from Minneapolis Convention | | 350.84 | |
| Miscellaneous Income | | 7.20 | |
| Total Net Receipts 1937 | | | 2,348.20 |
| "Cereal Laboratory Methods" Sales during 1937 | | 387.90 | |
| Interest on Invested Funds | | 9.30 | |
| Total Net Receipts 1937 | | | 397.20 |
| Decennial Index | | | |
| Received from Cereal Chemistry and Association | | | 162.50 |
| TOTAL RECEIPTS OF ALL ACCOUNTS 1937 | | | $11,140.47 |

DISBURSEMENTS

| | | | |
|---|---|---|---|
| Cereal Chemistry | | | |
| Cost of printing Journal and Reprints | $7,255.97 | | |
| 1937 Accounts Payable | 63.83 | | |
| Less: 1936 Accounts paid in 1937 | *1,158.70* | | |
| Net Cost of Printing | | $6,161.10 | |
| Cost of Editing and Miscellaneous Expenses | | 1,690.89 | |
| Reprinting of Volume I, No. 1 Cereal Chemistry | | 155.00 | |
| Decennial Index—Cereal Chemistry's 1937 Assessment | | 81.25 | |
| Net Disbursements 1937 | | 8,088.24 | |
| Surplus 1937 | | | $ 144.33 |
| Association | | | |
| Expenses of President's, Vice-President's Offices and News Letter | | 349.58 | |
| Expenses of Secretary's Office | | 258.59 | |
| Expenses of Treasurer's Office | | 198.36 | |
| Committee Expenses | | 69.58 | |
| Minneapolis Convention Report | | 316.90 | |
| Decennial Index—Association's 1937 Assessment | | 81.25 | |
| Expenses of Kansas City Exhibit | | 55.91 | |
| Miscellaneous Expense | | 27.00 | |
| Net Disbursements 1937 | | 1,357.17 | |
| Surplus 1937 | | | 991.03 |
| "Cereal Laboratory Methods" | | | |
| Printing and Mailing Expenses | | 104.65 | |
| Surplus 1937 | | | 292.55 |
| Decennial Index | | | |
| Surplus 1937 | | | 162.50 |
| TOTAL DISBURSEMENTS OF ALL ACCOUNTS | | $9,550.56 | |

## DISTRIBUTION OF NET ASSETS

| | | |
|---|---|---|
| Cereal Chemistry Assets 1936 | $3,829.74 | |
| Surplus 1937 | 144.33 | |
| Interest Earned 1936—Not previously entered | 7.50 | |
| Assets December 31, 1937 | | $3,981.57 |
| Association Assets 1936 | 3,156.82 | |
| Surplus 1937 | 640.19 | |
| Interest Earned 1936—Not previously entered | 21.53 | |
| Assets December 31, 1937 | | 3,818.54 |
| Convention Reserve Fund 1936 | 649.16 | |
| Surplus 1937 | 350.84 | |
| Assets December 31, 1937 | | 1,000.00 |
| Cereal Laboratory Methods Fund 1936 | 777.74 | |
| Surplus 1937 | 292.55 | |
| Assets December 31, 1937 | | 1,070.29 |
| Decennial Index Fund 1937 Deficit | *162.50* | |
| Surplus 1937 | 162.50 | |
| Assets December 31, 1937 | | None |
| Experimental Laboratory Baking 1936 | 80.95 | |
| Assets December 31, 1937 | | 80.95 |
| TOTAL ASSETS DECEMBER 31, 1937 | | $9,951.35 |

## FINANCIAL STATEMENT DECEMBER 31, 1937

### ASSETS

| | |
|---|---|
| Manufacturers Trust Co.—Checking Account | $3,481.91 |
| Petty Cash Fund—Washington, D. C. | 200.00 |
| Emigrant Industrial Savings Bank—New York | 834.44 |
| Franklin Savings Bank—New York | 511.78 |
| Harris Trust Company—Chicago | 1,987.05 |
| Building and Loan Stock—Kansas City [1] | 1,000.00 |
| U. S. Treasury Bonds | 2,000.00 |
| GROSS ASSETS | $10,015.18 |

### LIABILITIES

| | |
|---|---|
| 1937 Accounts Payable | 63.83 |
| NET ASSETS | $9,951.35 |

## REPORTS OF THE AUDITING COMMITTEES

We have examined the books and the report of the Treasurer for the year 1937 and to the best of our knowledge and belief, these are a true and accurate account of the receipts and expenditures of the American Association of Cereal Chemists.

H. K. PARKER, *Chairman*
CHAS. A. GLABAU
W. E. STOKES

We have examined the books of the Managing Editor of CEREAL CHEMISTRY for the calendar year 1937 and find the same to be correct to the best of our knowledge.

J. A. LECLERC
L. H. BAILEY

[1] Carried on the books at 50% of face value by order of Executive Committee of 1936 pending liquidation of Bankrupt Association.